Metabolomic approach to study the impact of flour type and fermentation process on volatile profile of bakery products

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A B S T R A C T

Metabolomic approaches applied to fermented foods are at the state of the science and represent a robust and reliable approach to identify, quantify and characterise the biochemical profiles of raw materials and transformed products. The outcomes so far obtained are cornerstones to understand mainly nutritional and sensorial inherent features. Formulations of new bakery products with increased nutritional values is trending the market, but sensorial attributes still need to be improved to reach a wider audience. The present work describes the application of gas chromatography–mass spectrometry (GC–MS) and electronic nose analyses, to investigate over the volatilome of different bakery products, obtained from mature and immature grains (KAMUT® khorasan and durum wheat) and transformed by a sourdough made of Lactobacillus spp. and *Saccharomyces cerevisiae*.

From the recipient results has emerged that the sensors used can distinguish the KAMUT® khorasan doughs fermented industrially at the fully ripe stage, the same doughs at the milky stage and KAMUT® khorasan sourdough at the fully ripe stage. Electronic nose allowed discriminating between different types of flours and GC–MS indicated the volatilome of sourdough KAMUT® khorasan case as the most promising. Thus, the combination of different independent variables in the bread process to improve the sensorial quality of the product, when is backed by metabolomics, represents an effective approach to study, characterise and exploit the sensorial quality of breads.

Keywords:
Kernel maturation stage
Sourdough
KAMUT® khorasan
SPME-GC–MS
Electronic nose
Metabolomics
Fermentation

1. Introduction

The taste and aroma are very important attributes for the food industry, especially in bakery and pastry industry. These issues are mainly featured by alcohols, ketones, aldehydes and acids as part of the volatile organic compounds, known as the volatilome. The search for new formulations in bakery products, in order to better structure, nutritional value and aroma, is continuously faced by food scientists, from breeders to biotechnologists. Moreover, the search for new bakery products with higher nutritional values is lagging behind the exploitation of sensorial features. Indeed, this kind of products still has pretty less attractive structural and sensorial qualities in comparison to standard products, e.g. those made with durum wheat. In instance, aiming to enhance sensorial attributes of breads, it is possible to combine and test different independent variables, as plant genotype, plant maturation stage, fermentation and baking processes. Among genotype tetraploid wheat varieties alternatives to durum wheat Claudio, KAMUT® khorasan is one of the most known, and recently the use of KAMUT® Khorasan as an ingredient in bread making has been widely studied with positive results either for his nutritional or technological properties (F. Balestra et al., 2015; Di Renzo, Reale, Boscaino, & Messia, 2018). Otherwise, sensorial properties of KAMUT® khorasan based bakery products remain poorly described.

Considering the maturation stage of the plant, it is known that: immature grains are usually rich in dietary fibre, whereas mature grains contain a high concentration of starch and a low concentration of fibre (Saa, Di Silvestro, Dinelli, & Gianotti, 2017). Therefore, the use of immature grain as ingredient may represent a good alternative to use in formulation of new products in the bakery industries (Skogerson et al., 2010; Verspreet et al., 2013), and is expectable that immature grains, that harbour a specific volatilome from that of fully ripped grains, shall confer different sensorial quality to doughs and breads.

The fermentation and baking processes can influence the flavour of fermented foods in different ways. During a lactic acid fermentation, usually the most evident change is the production of acid that lowers the pH, thus increasing the sourness. Given that most of the acid produced during fermentation will derive from the metabolism of sugars, sweetness will probably decrease as sourness increases (McFeeters,

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2004). It has been demonstrated that sourdough microflora, mainly lactic acid bacteria (LAB) and yeasts, have important metabolic interactions contributing to a production of flavour compounds including volatiles (De Vuyst & Neysens, 2005).

In fact, prolonged dough fermentation generates intense proteolysis and higher amounts of free amino acids that can operate as precursors of Strecker aldehydes mostly responsible for 'malty' odor note, such as methylpropanal, 2- and 3-methylbutanal, and methional (Zehentbauer & Grosch, 1998). For example, long fermentation time increases the concentration of 3-methyl-butanol and 2-phenyl-ethanol (Å. Hansen & Hansen, 1996). These compounds are directly related to the fermentative activity of the yeast during dough fermentation.

Benady and co-authors have used electronic olfactory systems to evaluate the fruit maturation and shelf-life consisting of sensing the aromatic volatiles emitted (Benady, Simon, Charles, & Miles, 1995). Analysis of VOCs (volatile organic compounds) in cereals and derived products by means of an olfactory system has been applied with increasing occurrence in food science research. (Falasconi et al., 2012; Sapirstein, Siddhu, & Aliani, 2012). Even though this kind of analysis focuses mostly on mycological safety issues, it can be used as an important tool to improve the sensory attributes of cereal products. Metabolic profiling requires the analysis of metabolites groups associated either to a specific metabolic pathway or to comprising a class of compounds (Dettmer, Aronov, & Hammock, 2007; Mozzi, Ortiz, Bleckwedel, De Vuyst, & Pescuma, 2013). In fermented foods, the metabolite profiling is applied to observe metabolite modifications during fermentation and to predict the sensorial and nutritional quality of final product (Mozzi et al., 2013). The aim of this study was to investigate the metabolite profiling of KAMUT® Khorasan and durum wheat made from immature grain by using gas chromatography mass spectrometry (GC–MS) and electronic nose during a sourdough fermentation.

2. Material and methods

2.1. Flours

KAMUT® khorasan grain (commercial ancient grain Khorasan, *T. turgidum* ssp. *turanicum* Jakubz. usually sold under the brand name of KAMUT®, which contains a high concentration of protein (Table 1) and durum wheat grain (cv. Claudio, [*T. turgidum* ssp. *durum* (Desf.)Huns.]) were obtained from the Department of Agricultural Sciences, University of Bologna (Italy). Wheat samples were grown at the same location during the same growing season. Grains were collected at the milky (75–79 BBCH scale; 15 days after anthesis) and full ripe maturity stages (89 BBCH scale). Wheat samples were air dried until the 12% humidity was reached and stone milled (100% flour extraction).

2.2. Strains used and growth media

Lactobacillus plantarum 98a, *Lactobacillus sanfranciscensis* BB12, *Lactobacillus brevis* 3BHI and *Saccharomyces cerevisiae* LBS strain, used in this work belong to the Department of Agricultural and Food Science and Technology (DISTAL) of Bologna University (Italy). LAB strains were grown separately in the Man Rogosa Sharpe (MRS) broth (Oxoid, Milan, Italy) at 37 °C for 24 h and the *Saccharomyces cerevisiae* strain was grown in the yeast extract peptone dextrose (YPD) broth at 28 °C for 24 h. The cells have been harvested by centrifugation 4000 *g*, for 10 min, and washed twice with sterile water.

2.3. Fermentation and baking processes

To prepare sourdough (SOUR) 600 g of KAMUT® khorasan and durum wheat flour were gently mixed with 270 mL of water, inoculated with 80 mL of each strain separately grown and incubated at 30 °C for 24 h.

In the doughs levels of inocula were approximately 4×108 CFU/g for LAB and 3×106 CFU/g for *S. cerevisiae*.

To prepare the industrial fermentation (IND), considered as the reference case, 600 g of both flours were mixed with 270 mL of water, inoculated with 180 mL of *S. cerevisiae* (3×10^6 CFU/g) and incubated at 30 °C for 90 min. The fermented doughs were baked in two different ways: i) long time/high temperature (250 °C for 20 min) and ii) short time/low temperature (210 °C for 10 min).

2.4. pH analysis

Ten grams of dough were blended using a laboratory blender with 90 mL of deionised water for 2 min. pH values of the solutions were recorded in triplicate using a pH-meter (BASIC 20, CRISON mod-334-B).

2.5. Electronic nose (E-nose)

An electronic nose device PEN2, provided by (WMA Airsense Analysentechnik GmbH) Schwerin, Germany, was used. The portable electronic nose PEN2 has an array of 10 different metal oxide sensors (Table S1 describes the properties of sensors used) positioned into a small chamber (V = 1.8 mL). Seven grams of dough were placed in 10 mL vials and the vials were sealed. The headspace inside the vials was equilibrated for 20 min at 25 °C. The headspace gas was pumped over the sensors of the electronic-nose with a flow of 400 mL/min. During the measurement phase, the bomb detector pushes the volatiles though a closed loop that includes the measurement and concentration chambers. No air enters or exits the loop. The measurement phase lasts 120 s, enough time for sensors to reach a stable value. The collected data interval was 1 s. The sampling temperature was 25 °C and injection temperature was 180 °C. Bread samples have not been analysed with E-nose.

2.6. Solid-phase microextraction-gas chromatography–mass spectrometry (SPME-GC–MS) analysis

A polydimethylsiloxane-divinylbenzene-carboxen coated fibre (DVB-carboxen/PDMS, 50/30 μm) and a manual SPME holder (Supelco

Table 1
Characterisation of the flour obtained from milky and full ripe grain. Protein and dietary fibre (IDF, SDF, TDF) contents are expressed as g/100 g DW (± standard deviation), while polyphenol and flavonoid contents are expressed as mg/100 g DW (± standard deviation).

	Claudio (*T. turgidum* ssp. *durum*)		KAMUT® Khorasan wheat	
	Milky	Fully ripe	Milky	Fully ripe
Protein	11.6 ± 0.1	10.5 ± 0.1	13.4 ± 0.1	11.2 ± 0.1
IDF	14.6 ± 0.3	13.0 ± 0.9	13.6 ± 0.1	11.4 ± 0.1
SDF	4.5 ± 0.4	4.2 ± 0.3	5.5 ± 0.3	4.8 ± 0.1
TDF	19.1 ± 0.1	17.3 ± 0.7	19.2 ± 0.2	16.3 ± 0.1
Total polyphenols	232.1 ± 1.6	259.1 ± 2.1	252.7 ± 2.8	274.4 ± 0.5
Total flavonoids	48.6 ± 1.4	42.0 ± 1.7	32.5 ± 2.9	37.7 ± 0.3

Table 2
pH values * at the beginning (T0) and at the end of the industrial (IND) and sourdough fermentation (SOUR).

Samples	T0	IND	SOUR
RW	6.48 ± 0.13	6.25 ± 0.02	3.84 ± 0.03
MW	6.45 ± 0.14	6.22 ± 0.01	3.81 ± 0.01
MK	6.58 ± 0.05	6.45 ± 0.02	3.83 ± 0.00
RK	6.34 ± 0.04	6.30 ± 0.01	3.81 ± 0.01

Legend: RW: durum wheat fully ripe; MW: durum wheat milky stage; RK: KAMUT® khorasan fully ripe; MK: KAMUT® khorasan milky stage.
* Mean ± sd of two replicates.

Inc., Bellefonte, PA, USA) were used after preconditioning according to the manufacturer's instruction manual. GC–MS analysis was carried out on an Agilent 7890A Gas Chromatograph (Agilent Technologies, Palo Alto, CA, USA) coupled to an Agilent 5975C mass selective detector operating in electron impact mode (ionization voltage of 70 eV). A Chrompack CP-Wax 52 CB capillary column (50 m length, 0.32 mm ID) was used (Chrompack, Middelburg, The Netherlands). Before each head space sampling, the fibre was exposed to the GC inlet for 10 min for thermal desorption at 250 °C in a blank sample. Five grams of dough or bread were minced and placed in 10 mL vials and sealed. The samples were then equilibrated for 10 min at 50 °C. The SPME fibre was exposed to each sample for 40 min and finally the fibre was inserted into the injection port of the GC for a 10 min sample desorption. The temperature program was: 50 °C for 0 min then programmed at 1.5 °C/min to 65 °C and finally at 3.5 °C/min to 220 °C, which was maintained for 20 min. Injector, interface and ion source temperatures were 250, 250 and 230 °C, respectively. Injections were carried out in splitless mode and helium (3 mL/min) was used as carrier gas according to Serrazanetti et al. (2011).

All GC–MS raw files were converted to netCDF format via Chemstation (Agilent Technologies, Palo Alto, CA, USA) and subsequently processed with the XCMS toolbox (http://metlin.scripps.edu/download/) for automatic and simultaneous retention time alignment, matched filtration, peak detection, and peak matching. The resulting table containing information such as peak index (retention time-*m/z* pair) and normalized peak area was exported into R 3.0.0 (www.r-project.org) for subsequent statistical analysis (Saa et al., 2014; Serrazanetti et al., 2011).

Identification of molecules was carried out by comparing their retention times with those of pure compounds (Sigma-Aldrich, Milan, Italy), and confirmed by searching mass spectra in the available databases (NIST version 2005 and Wiley version 1996) and literature. No internal standard was used because the analysis is qualitative and the relative abundance of the volatile compound is expressed as (compound peak area/total compounds peak area) × 100 in each sample (Supplementary data).

2.7. Statistical analysis

Data for the E-nose analysis for a given sample corresponds to the mean of all sensors that were statistically significant from at least four replicates, the canonical analysis of principal coordinates (CAP) analysis was applied to these data sets.

The SPME-GC–MS analysis has been done with at least two replicates. After the identification, ANOVA analysis was performed and only the molecules statistically significant were select for the CAP analysis.

The CAP was performed in the R environment using the vegan package to discriminate between and within the dataset based on the metabolomic data (Di Cagno et al., 2011). The results of this analysis give two different orthogonal matrices which can be plotted on n-dimensional space based on the number of canonical axis calculated: the first, called the "CAP score plot", illustrates a summary of the relationship among the observations (or samples), and the second matrix "loading score plot" shows a summary of the variables (properties) that gives an idea of which molecules discriminate more between the different group of the score plot. The loading plot can also represent the weights for each original variable when calculating the canonical axis.

The first canonical axis has the highest variance, and the second one has the second highest variance and so on. The values of the score plot will be on the negative or positive pole of the canonical axis 1, which has the highest variance. So looking at the direction of each molecule on this axis, we can identify the discriminant molecules in our dataset.

3. Results

3.1. pH

The pH of the IND samples obtained with durum wheat flour (both MW and RW) passed from the initial 6.4 to 6.2 after 1.5 h. On the other hand, the KAMUT® khorasan IND doughs were characterized by a pH of 6.5 and 6.34 for MK and RK respectively at T0. After 1.5 h of fermentation, they decreased to 6.45 and 6.30 respectively. After 24 h, the SOUR samples made with durum wheat and KAMUT® khorasan flour had an average pH value of 3.80 (Table 2).

3.2. Electronic nose

A CAP analysis has been performed on the E-nose data analysis of dough. The results of the score plot of the first two components or canonical axis are shown on Fig. 1A, and from this latter, the first component or the canonical axis 1 (CAP1), which gave the major percentage of explained variation (76.73%) has been extracted as illustrated on Fig. 1B. The genotype - fermentation process has not been taking into account here because the discrimination was not clear between sourdough and industrial fermentation as it was between fully ripe and milky stage (supplement Fig. 1). In Fig. 1B, it can be observed that the E-nose could distinguish the maturation stage and the genotype. The classification is clearer looking only at the genotype. So based only on the canonical axis 1, the sensors were able to separate four distinct clusters according to the cereal genotype and the maturation stage. Along the negative pole of canonical axe 1, the KAMUT® khorasan samples are represented: both fully ripe and milky, while on the line (± 0) the durum wheat fully ripe counterparts is found. As the samples are separated between positive and negative values which are the direction of each variable in this first component. In general, samples on the negative side has high value of sensors on that same side because the loading and the score plot overlapped. The loading coefficient or sensors that are discriminating in this dataset based on the maturation and genotype were plotted in Fig. 1C. At the negative pole, the sensors mainly responsible of the discrimination are S3 (aromatic), S5 (aromatic-aliphatic), S1 (aromatic), S7 (sulphur-organic) whereas at the positive pole S2 (broad-range), S10 (methane-aliphatic), S4 (hydrogen) are found. On the other hand, the sensors that have less influence, are S9 (sulphur-chlor) and S6 (broad-methane) at the positive axe and S8 (broad-alcohol) at the negative.

3.3. SPME-GC–MS analysis

Up to 75 molecules belonging to the families of alcohols, aldehydes, ketones, carboxylic acids, esters, phenols, organic acids, and hydrocarbons were identified in dough samples. Using the same approach of E-nose data analysis, the first component of the CAP has been extrapolated. Fig. 2A represents the loading plot of the CAP1, which explained 57.20% of the total variation of the dough samples dataset.

In general, the pool of volatile compounds appeared to be different between KAMUT® khorasan and durum wheat dough independently of the fermentation and maturation stage. The discrimination genotype – maturation and genotype – fermentation (Supplement Fig. 2) gives the

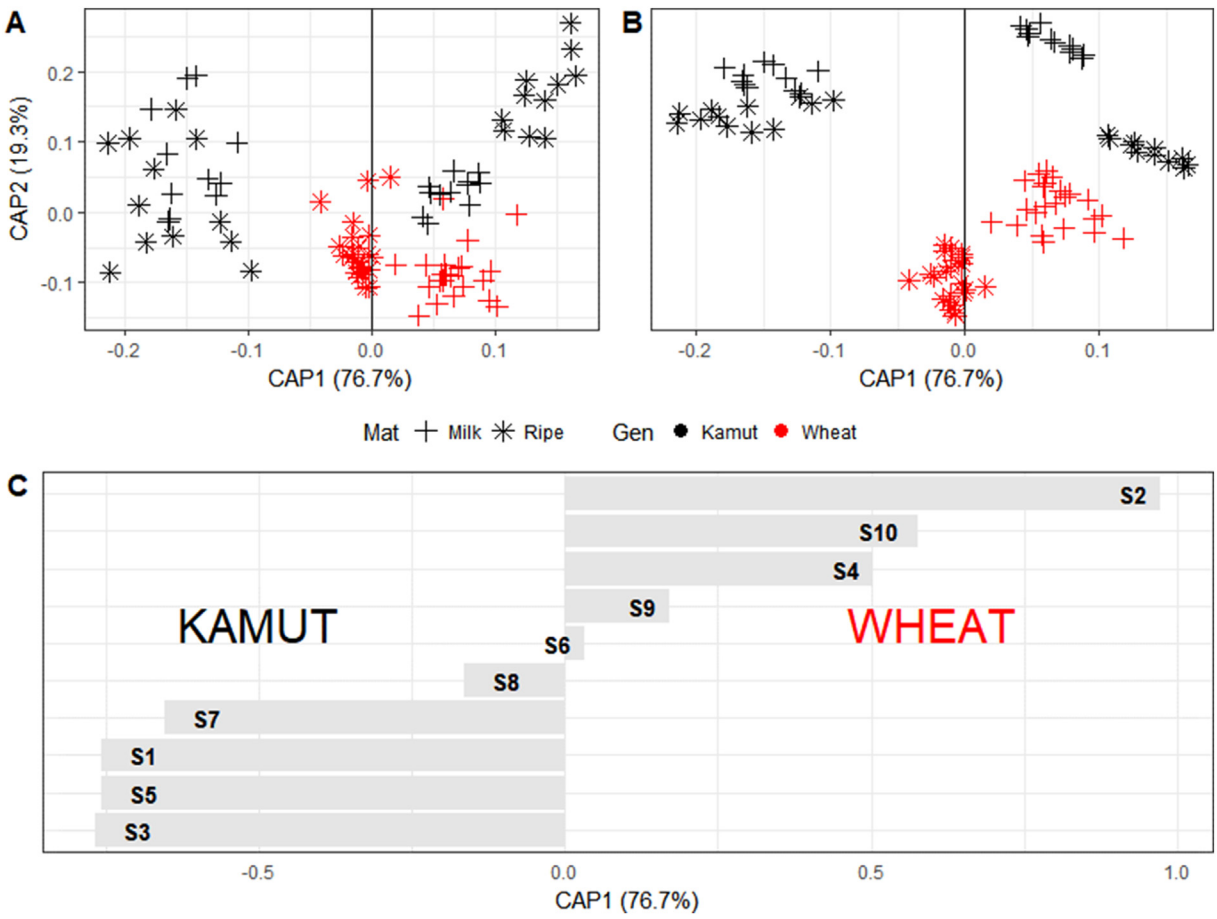

Fig. 1. E-nose dough sample; A: 2D CAP score plot of KAMUT® Khorasan (black) vs. durum wheat (red) and milky stage vs. ripe. B: 1D CAP1 score plot of KAMUT® Khorasan (black) vs. durum wheat (red) and milky stage vs. ripe. C: CAP1 loading plot of Kamut® khorasan vs. durum wheat. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

same distribution on CAP1. Here, the discrimination genotype – maturation was plotted to better explain the loading coefficients (Fig. 2A). The pool of KAMUT® khorasan dough's volatile compounds is along the negative pole of CAP1 as showed in Fig. 2B and the molecules released were mainly alcohols (1-hexanol)), aldehyde like 2-butyl-2-octanal and carboxylic acids (3-methyl pentanoic acid, p-tert-butyl benzoic acid, acetic acid and hexyl ester acetic acid).

Concerning durum wheat which molecules are along the positive pole of axis 1 (Fig. 2B), molecules like 2,5-dimethylfuran, aldehydes (hexanal, decanal), aromatic hydrocarbon compounds (ethylbenzene, p-xylene), and some hydrocarbons compounds (3-ethyl-2-methyl-1,3-hexadiene) were detected. It appears that the KAMUT® khorasan dataset had a broad range and more volatile compounds compared to durum wheat. In fact, on the table S2 where are reported the relative abundances of the identified molecules, it is shown that 1-hexanol is higher in KAMUT® Khorasan whereas 2, 5-dimethylfuran is higher in durum wheat.

The total number of volatile compounds (60 molecules) quantified in baked samples was less than the fermented ones (about 75 molecules). As illustrated on Fig. 3A industrially baked breads are well separated from sourdough-baked breads along the canonical axis 1 that explained 51.30% of the total variation. The separation genotype – maturation (supplement fig. 3) was not clear along this axis contrary to what it has been observed in doughs. Observing the Fig. 3A, the CAP1 allows a good separation of baked samples based on the maturation – fermentation. Fig. 3B shows the volatile compounds that discriminated the baked fermented samples along CAP1. Ethyl alcohol, 3-hydroxy-2-butanone, acetic acid, octanoic acid, heptanoic acid, 3-methyl butanoic acid are the compounds principally responsible for the discrimination of sourdough breads whereas heptanal, acetophenone, ethylbenzene, 2-methyl-3-decen-5-one, 2,4-bis (1-methylethyl) phenol, are those responsible of industrial fermentation discrimination. On the table S3 that reported the relative abundances of each identified metabolites, it is observed that ethyl alcohol is higher in sourdough breads whereas heptanal is higher in industrial ones.

The CAP analysis was not able to separate the time-temperature cycle (data not shown).

4. Discussion

It can be noted that both methods (E-nose and SPME-GC–MS) were able to discriminate between KAMUT® khorasan and durum wheat when the samples used was raw material (dough). From the E-nose results, it emerged that these sensors can clearly distinguish durum wheat at the fully ripe stage from milky. The E-nose was also able to classify the KAMUT® khorasan alone both milky stage and fully ripe stage. From the sensors, it is shown that the KAMUT® khorasan is more aromatic than wheat, Di Renzo et al. (2018) confirmed that on their study. Some authors reported that the grain composition could differ according to the stage of kernel development and to the specific types of processing applied (Skogerson et al., 2010; Verspreet et al., 2013). Actually, as expected, different flours produce different volatile profiling accordingly to their role as substrate for the LAB fermentation. KAMUT® khorasan has more protein than wheat (Table 1), leading to more differentiation of sulphuric compounds (S7). This result confirms a previous study by Balestra et al. (2015) where the use of E-nose gave

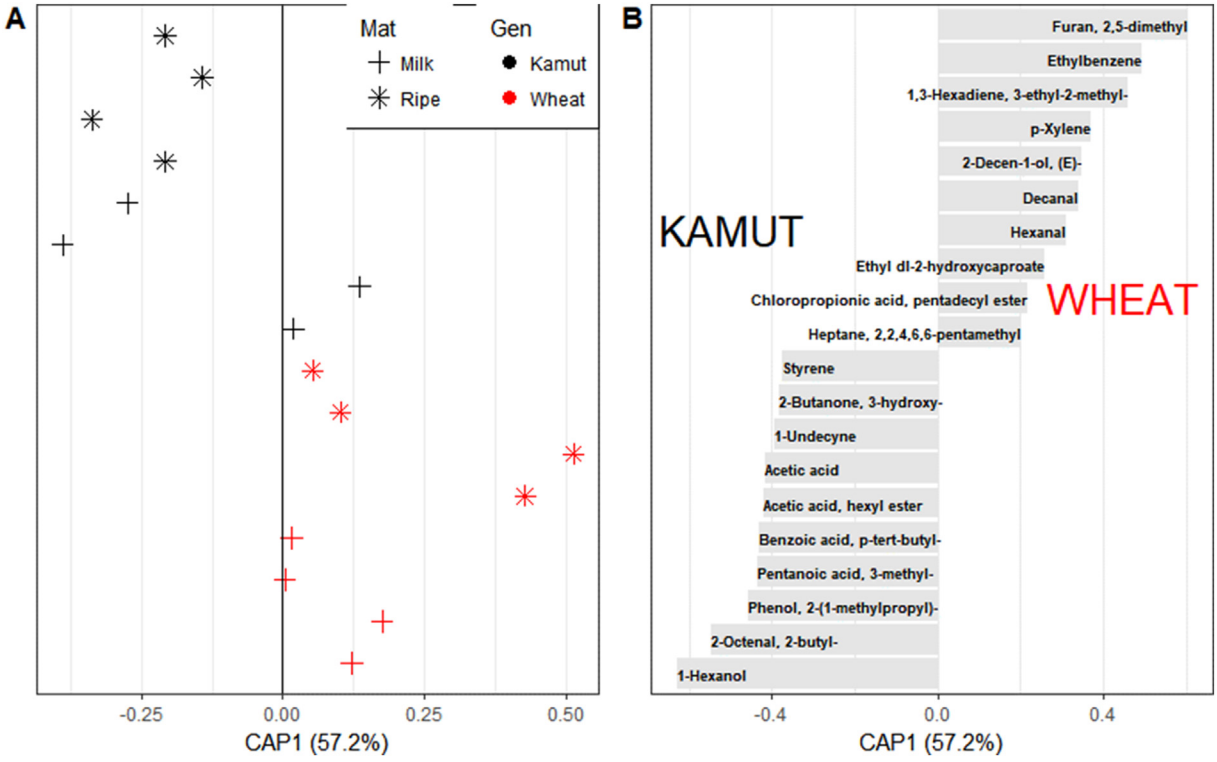

Fig. 2. A: CAP1 score plot of KAMUT® Khorasan (black) vs. durum wheat (red) and milky stage vs. ripe dough samples using SPME-GC–MS. B: CAP1 loading plot of Kamut® khorasan vs. durum wheat dough samples using SPME-GC–MS. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

good results in the discrimination of KAMUT® khorasan samples from durum wheat samples fermented with yeast but with a less extent in chemical acidified doughs. Several studies reported positive applications of electronic nose technology to the discrimination of fruit of different qualities, with tomatoes (Berna, Lammertyn, Saevels, Di Natale, & Nicola, 2004), oranges (Gómez, Wang, Hu, & Pereira, 2006),

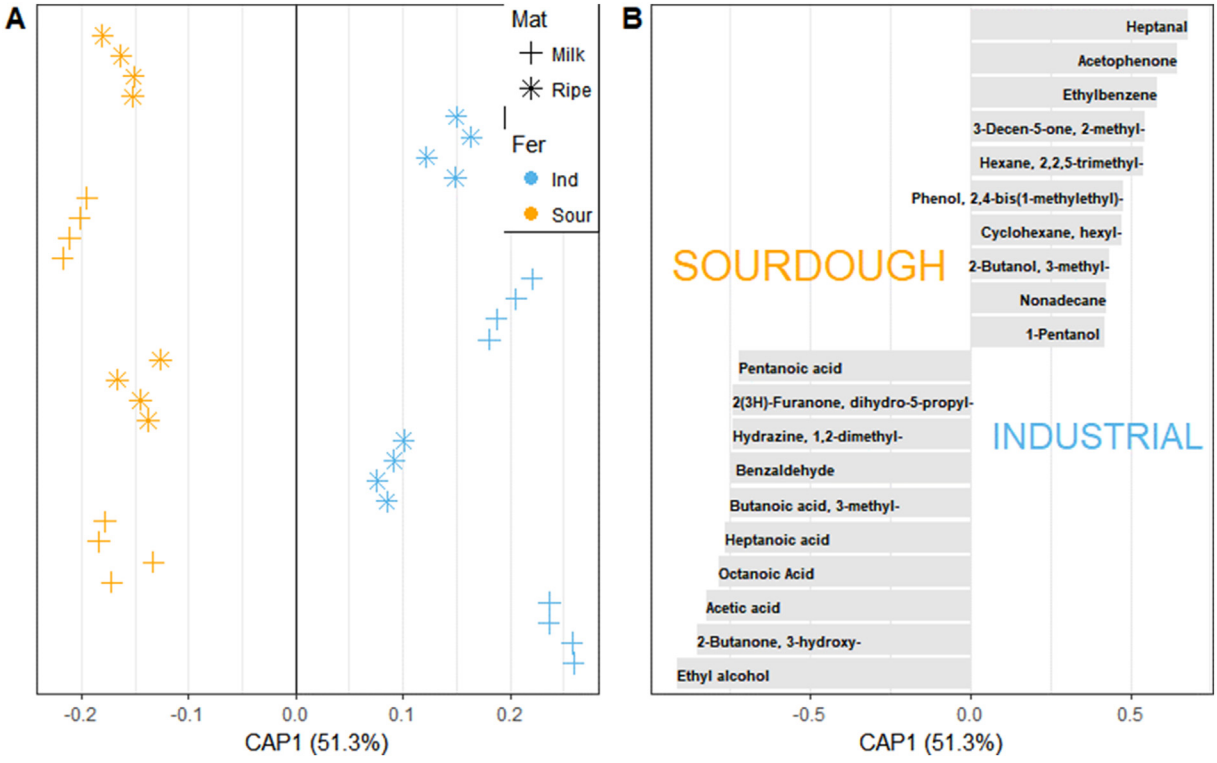

Fig. 3. A: CAP1 score plot of sourdough (orange) vs. industrial or yeast fermentation (blu) and milky stage vs. ripe bread samples using SPME-GC–MS. B: CAP1 loading plot of sourdough vs. industrial or yeast fermentation bread samples using SPME-GC–MS.

apples (Saevels et al., 2003) and cherries (Toivonen, Kappel, Stan, McKenzie, & Hocking, 2006) but till now few literatures refer to cereals if not only for a mycology aspect (Wang, Wang, Liu, & Liu, 2012).

The pool of volatile compounds of the KAMUT® khorasan samples identified using the SPME-GC–MS were mainly from sourdough fermentation. It is known that the primary metabolic products of LAB are lactic acids and acetic acids responsible for the sensory qualities of sourdough bread with citric and malic acids that are also produced by LAB but in lesser amounts (Kam, AIDA, Sahilah, & Maskat, 2011; Yazar & Tavman, 2012). Therefore, the production of acetic acid as the major discriminant acid in KAMUT® khorasan is probably the result of the interactions between flour nutrients, naturally occurring microflora and sourdough LAB. This result demonstrates that KAMUT® Khorasan is a good substrate for LAB fermentation.

The 2-butyl-2-octenal which is discriminating in KAMUT® khorasan dough is generated by the fatty acid composition followed by the condensation of aldehydes products and like 2-propyl-2-octenal, and lipid oxidation products such as (E)- and (Z)-3-octene-2-one, (E, E)- and (E, Z)- 3, 5-octadien-2-one it may also be responsible for the rancid flavour (Heiniö, Lehtinen, Oksman-Caldentey, & Poutanen, 2002). The high quantity of hexanol found in KAMUT® Khorasan is in agreement with Di Renzo et al. (2018).

The presence of ethanol found in sourdough is also in agreement with others studies (Kam et al., 2011). These authors demonstrated that ethanol and ethyl acetate were generated in the highest amounts in sourdough fermented by *Lb. sanfranciscensis*, while ethyl-n-propanoate, butyl-acetate and n-pentyl acetate were only produced in sourdough started with yeasts. The effect of the microbial activity on the volatile compound synthesis have been well studied (Meignen et al., 2001). Schieberle (1996) showed that alcohols can be produced by yeast via the Ehrlich pathway by transamination of some amino acids into the corresponding α-keto acids, followed by a decarboxylation into the aldehyde and finally reduction into the alcohol, explaining the detection of alcohol like 3-methyl-2-butanol in industrial fermentation. The production of hexanal, heptanal and decanal in wheat is usually due to the oxidative degradation of unsaturated fatty acids (Beleggia, Platani, Spano, Monteleone, & Cattivelli, 2009). In our samples, the relative abundance of hexanal is high in the wheat fully ripe industrially fermented compared to others (Table S1). Heptanal, hexanal and decanal are found at the high relative abundance in the KAMUT® khorasan industrially fermented breads, probably is the result of enzymatic or thermal oxidation of lipids. In addition, the relative abundance of 3-methyl-2-butanol, which is probably produced by yeast via the Ehrlich pathway by transamination of amino acids, is higher in KAMUT than wheat (Table S2).

The KAMUT® khorasan samples was also characterized by the presence of short chain fatty acids (SCFA) like acetic acid and pentanoic acid, making it a good candidate for the production of cereal foods with high nutritional value (Saa et al., 2014). The sourdough fermentation influences bread quality, extensibility, softness, volume and activity of endogenous enzymes, so it is important to select good ingredients to enhance these properties.

Beleggia et al. (2009) in their study detected some metabolites at high concentrations in durum wheat grain, which significantly distinguished this cereal from the others, including butyrolactone, alpha-pinene, propylbenzene, 1-ethyl-3-methylbenzene and cymene. They confirm our findings as ethyl benzene highly discriminated durum wheat fermented samples. Even the presence of benzene derivatives and terpenes has been found significantly higher in wheat durum as previously identified by others authors (Busko et al., 2010). Lee, Alexander, and Jonnalgadda (2013) concluded that processing may influence the metabolic activity of individual grains and grain fractions using a non-targeted metabolomic approach. Their study is in agreement with our outcomes as the different clusters based on the cereal genotype (specially at the fully ripe), are the consequence of the impact of the fermentation/baking process in combination with the genotype and maturation variables. According to Poinot et al. (2008) baking process generates Maillard volatile products, such as 2-methylpropanal, 2-methylfuran, 2-acetylfuran, 3-methylbutanal, 2,3-pentanedione, 1-methylpyrrole, 5-methyl-2-furfural, 2-meth-ylpyrazine, 2,3-dimethylpyrazine, pyrazine. None of these products has been found on the baked bread, meaning that in this study the time-temperature cycle did not have an influence on the volatile compounds.

Consumers are searching for products with good tasting and nutritional properties. Sourdough is mainly used to improve quality, taste and flavour of baking products, SPME-GC–MS was able to differentiate sourdough from industrial fermentation, so industries can used it to select and choose the right fermentation process. E-nose shows that KAMUT® khorasan is more aromatic than wheat, proving that it may be used to quickly verify the type of a genotype.

5. Conclusion

The application of olfactometry system such as E-nose can be useful in food industry but it is important to consider the sensors specificity and suitability for that food matrix. Food industries are looking for bakery products with aromatic and nutritional properties. In this paper, using the E-nose it was possible to discriminate the presence of the two type of flours in order to control and monitor the bread making process. Because each flour has distinct aromatic properties, it is important to know it to obtain the desired flavour compounds. As a first step for a baking process, a rapid analysis can be done with E-nose to verify the aromatic compounds of each flour and control if the flour is the right type. With dough samples, it was possible to separate the different genotype maturation and fermentation with E-nose and SPME-GC–MS. Most of the volatile compounds including SCFA, identified by SPME-GC–MS on the sourdough were mainly from the KAMUT® khorasan dough samples making it a good candidate for the production of bakery production with high nutritional and healthy value. Sourdough process improve the organoleptic and nutritional properties of cereal-fermented foods. The approach used here, may represent a valuable tool to both control and optimize a sourdough process. In fact, SPME-GC–MS shows that sourdough process generates different volatile compounds compared to industrial fermentation. It can be also a useful tool aimed to select the most promising combination of raw materials and fermentation processes for the production of bakery products with immature grains having high nutritional value using sensory evaluation and GC–MS.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodres.2019.01.024.

References

Balestra, F., Laghi, L., Saa, D. T., Gianotti, A., Rocculi, P., & Pinnavaia, G. (2015). Physico-chemical and metabolomic characterization of Kamut® Khorasan and durum wheat fermented dough. *Food Chemistry*.

Beleggia, R., Platani, C., Spano, G., Monteleone, M., & Cattivelli, L. (2009). Metabolic profiling and analysis of volatile composition of durum wheat semolina and pasta. *Journal of Cereal Science, 49*(2), 301–309.

Benady, M., Simon, J., Charles, D., & Miles, G. (1995). Fruit ripeness determination by electronic sensing of aromatic volatiles. *Transactions of the ASAE, 38*(1), 251–257.

Berna, A. Z., Lammertyn, J., Saevels, S., Di Natale, C., & Nicola, B. M. (2004). Electronic nose systems to study shelf life and cultivar effect on tomato aroma profile. *Sensors and Actuators B: Chemical, 97*(2), 324–333.

Busko, M., Jelen, H., Goral, T., Chmielewski, J., Stuper, K., Szwajkowska-Michalek, L., ... Perkowski, J. (2010). Volatile metabolites in various cereal grains. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment, 27*(11), 1574–1581. https://doi.org/10.1080/19440049.2010.506600.

De Vuyst, L., & Neysens, P. (2005). The sourdough microflora: biodiversity and metabolic interactions. *Trends in Food Science & Technology, 16*(1), 43–56.

Dettmer, K., Aronov, P. A., & Hammock, B. D. (2007). Mass spectrometry-based metabolomics. *Mass Spectrometry Reviews, 26*(1), 51–78.

Di Cagno, R., De Angelis, M., De Pasquale, I., Ndagijimana, M., Vernocchi, P., Ricciuti, P., ... Guerzoni, M. E. (2011). Duodenal and faecal microbiota of celiac children: Molecular, phenotype and metabolome characterization. *BMC Microbiology, 11*(1), 219.

Di Renzo, T., Reale, A., Boscaino, F., & Messia, M. C. (2018). Flavoring production in Kamut®, quinoa and wheat doughs fermented by *Lactobacillus paracasei*, *Lactobacillus plantarum* and *Lactobacillus brevis*: A SPME-GC/MS study. *Frontiers in Microbiology, 9*, 429.

Falasconi, M., Concina, I., Gobbi, E., Sberveglieri, V., Pulvirenti, A., & Sberveglieri, G. (2012). Electronic nose for microbiological quality control of food products. *International Journal of Electrochemistry, 2012*.

Gómez, A. H., Wang, J., Hu, G., & Pereira, A. G. (2006). Electronic nose technique potential monitoring mandarin maturity. *Sensors and Actuators B: Chemical, 113*(1), 347–353.

Hansen, Å., & Hansen, B. (1996). Flavour of sourdough wheat bread crumb. *Zeitschrift für Lebensmittel-Untersuchung und Forschung, 202*(3), 244–249.

Heiniö, R.-L., Lehtinen, P., Oksman-Caldentey, K.-M., & Poutanen, K. (2002). Differences between sensory profiles and development of rancidity during long-term storage of native and processed oat. *Cereal Chemistry, 79*(3), 367–375.

Kam, W., AIDA, W., Sahilah, A., & Maskat, M. (2011). Volatile compounds and lactic acid bacteria in spontaneous fermented sourdough. *Sains Malaysiana, 40*(2), 135–138.

Lee, P., Alexander, D., & Jonnalgadda, S. S. (2013). Diversity of nutrient content in grains - a pilot metabolomics analysis. *Journal of Nutrition & Food Sciences, 03*(02), 191–193. https://doi.org/10.4172/2155-9600.1000191.

McFeeters, R. (2004). Fermentation microorganisms and flavor changes in fermented foods. *Journal of food science, 69*(1), FMS35–FMS37.

Meignen, B., Onno, B., Gélinas, P., Infantes, M., Guilois, S., & Cahagnier, B. (2001). Optimization of sourdough fermentation with *Lactobacillus brevis* and baker's yeast. *Food Microbiology, 18*(3), 239–245.

Mozzi, F., Ortiz, M. E., Bleckwedel, J., De Vuyst, L., & Pescuma, M. (2013). Metabolomics as a tool for the comprehensive understanding of fermented and functional foods with lactic acid bacteria. *Food Research International, 54*(1), 1152–1161. https://doi.org/10.1016/j.foodres.2012.11.010.

Poinot, P., Arvisenet, G., Grua-Priol, J., Colas, D., Fillonneau, C., Le Bail, A., & Prost, C. (2008). Influence of formulation and process on the aromatic profile and physical characteristics of bread. *Journal of Cereal Science, 48*(3), 686–697.

Saa, D. T., Turroni, S., Serrazanetti, D. I., Rampelli, S., Maccaferri, S., Candela, M., ... Gianotti, A. (2014). Impact of Kamut® Khorasan on gut microbiota and metabolome in healthy volunteers. *Food Research International, 63*, 227–232.

Saa, D. T., Di Silvestro, R., Dinelli, G., & Gianotti, A. (2017). Effect of sourdough fermentation and baking process severity on dietary fibre and phenolic compounds of immature wheat flour bread. *LWT-Food Science and Technology, 83*, 26–32.

Saevels, S., Lammertyn, J., Berna, A. Z., Veraverbeke, E. A., Di Natale, C., & Nicolaï, B. M. (2003). Electronic nose as a non-destructive tool to evaluate the optimal harvest date of apples. *Postharvest Biology and Technology, 30*(1), 3–14.

Sapirstein, H. D., Siddhu, S., & Aliani, M. (2012). Discrimination of volatiles of refined and whole wheat bread containing red and white wheat bran using an electronic nose. *Journal of Food Science, 77*(11), S399–S406.

Schieberle, P. (1996). Intense aroma compounds: Useful tools to monitor the influence of processing and storage on bread aroma. *Advances in Food Sciences, 18*(5–6), 237–244.

Serrazanetti, D. I., Ndagijimana, M., Sado-Kamdem, S. L., Corsetti, A., Vogel, R. F., Ehrmann, M., & Guerzoni, M. E. (2011). Acid stress-mediated metabolic shift in *Lactobacillus sanfranciscensis* LSCE1. *Applied and Environmental Microbiology, 77*(8), 2656–2666.

Skogerson, K., Harrigan, G. G., Reynolds, T. L., Halls, S. C., Ruebelt, M., Iandolino, A., ... Fiehn, O. (2010). Impact of genetics and environment on the metabolite composition of maize grain. *Journal of Agricultural and Food Chemistry, 58*(6), 3600–3610.

Toivonen, P., Kappel, F., Stan, S., McKenzie, D.-L., & Hocking, R. (2006). Factors affecting the quality of a novel fresh-cut sweet cherry product. *LWT- Food Science and Technology, 39*(3), 240–246.

Verspreet, J., Hemdane, S., Dornez, E., Cuyvers, S., Pollet, A., Delcour, J. A., & Courtin, C. M. (2013). Analysis of storage and structural carbohydrates in developing wheat (Triticum aestivum L.) grains using quantitative analysis and microscopy. *Journal of Agricultural and Food Chemistry, 61*(38), 9251–9259.

Wang, D., Wang, X., Liu, T., & Liu, Y. (2012). Prediction of total viable counts on chilled pork using an electronic nose combined with support vector machine. *Meat Science, 90*(2), 373–377.

Yazar, G., & Tavman, Ş. (2012). Functional and technological aspects of sourdough fermentation with *Lactobacillus sanfranciscensis*. *Food Engineering Reviews, 4*(3), 171–190. https://doi.org/10.1007/s12393-012-9052-1.

Zehentbauer, G., & Grosch, W. (1998). Crust aroma of baguettes I. Key odorants of baguettes prepared in two different ways. *Journal of Cereal Science, 28*(1), 81–92.