Analysis of volatile compounds in gluten-free bread crusts with an optimised and validated SPME-GC/QTOF methodology

Joana Pico [a*], Beatriz Antolín [a], Laura Román [b], Manuel Gómez [b], José Bernal [a]

[a] I.U.Cinquima, Analytical Chemistry Group, University of Valladolid, Paseo de Belén Street 7, E-47011 Valladolid, Spain.

[b] Food Technology Area, E.T.S. Ingenierías Agrarias, University of Valladolid, Madrid Avenue 57, E- 34071 Palencia, Spain.

*Corresponding author: Joana Pico e-mail: joana.pico@uva.es

Highlights

- ✓ A SPME-GC/QTOF method was developed and optimised for analysing bread crust aroma.
- ✓ SPME conditions implied 0.75 g of crust sample extracted at 60°C for 51 min.
- ✓ The proposed SPME-GC/QTOF methodology was sensible, precise, accurate and linear.
- ✓ Furfural was the most abundant compound in commercial wheat bread crust.
- ✓ Teff and wheat starch crusts showed contents of 2-ACPY close to wheat crust.

Abstract

The aroma of bread crust, as one of the first characteristics perceived, is essential for bread acceptance. However, gluten-free bread crusts exhibit weak aroma. A SPME-GC/QTOF methodology was optimised with PCA and RSM and validated for the quantification of 44 volatile compounds in bread crust, extracting 0.75 g of crust at 60°C for 51 min. LODs ranged between 3.60-1760 μgKg^{-1}, all the R^2 were higher than 0.99 and %RSD for precision and %Er for accuracy were lower than 9% and 12%, respectively. A commercial wheat bread crust was quantified, and furfural was the most abundant compound. Bread crusts of wheat starch and of japonica rice, basmati rice and teff flours were also quantified. Teff flour and wheat starch crusts were very suitable for improving gluten-free bread crust aroma, due to their similar content in 2-acetyl-1-pyrroline and 4-hydroxy-2,5-dimethyl-3(2H)-furanone compared to wheat flour crust and also for their high content in pyrazines.

Keywords: volatile compounds; SPME-GC/QTOF; bread crust; gluten-free bread; PCA; RSM.

Abbreviations: 2-ACPY (2-acetyl-1-pyrroline); CAR (carboxen); CCD (central composite design); D (desirability function); DVB (divinylbenzene); DOE (design of experiments); FD (flavour dilution factor); GC/QTOF (gas chromatography/quadrupole-time of flight); HPMC (hydroxyl propyl methyl cellulose); LOD (limit of detection); LOQ (limit of quantification); MSA (method of standard addition); OT (odour threshold); PA (polyacrylate); PC (principal component); PCA (principal component analysis); PDMS (polydimethylsiloxane); R^2 (coefficient of determination); Re (relative error); RSD (relative standard deviation); RSM (response surface method); SPME (solid-phase microextraction).

1. Introduction

The aroma of bread crust is one of the first attributes sensed when entering a bakery shop. It has been characterised by volatile compounds from Maillard reactions, caramelisation and thermal degradation (Pico, Bernal, & Gómez, 2015), although there can be volatile compounds from lipid oxidation in smaller proportions (Moskowitz, Bin, Elias, & Peterson, 2012). 2-Acetyl-1-pyrroline, generated by Maillard reactions, has been considered the key volatile compound of wheat flour bread crust. Other important volatile compounds include 3-methylbutanal, 2,3-butanedione and 4-hydroxy-2,5-dimethyl-3(2H)-furanone, also from Maillard reactions, along with 2-(E)-nonenal and 2,4-(E,E)-decadienal from lipid oxidation (Zehentbauer & Grosch, 1998).

In the case of gluten-free bread, the sensory quality is barely acceptable, almost notably the texture and the aroma (Pacyński, Wojtasiak, & Mildner-Szkudlarz, 2015). Quality parameters such as nutritional value, rheology of the dough, texture, volume and colour have been widely studied in gluten-free bread (Houben, Höchstötter, & Becker, 2012; Masure, Fierens, & Delcour, 2016). However, there is little knowledge regarding the aroma of gluten-free bread crusts. To our knowledge, only Pacyński et al. (2015) have studied the volatile compounds of gluten-free bread crusts with amino acid – sugar pairs added with the aim of promoting the generation of Maillard compounds and improving the aroma of the crust.

Therefore, the analysis of volatile compounds of bread crust becomes essential in order to improve bread quality, above all of gluten-free bread crusts. In the last decade, solid phase microextraction (SPME) combined with GC/MS has been preferred because it is a quick, simple and solvent-free technique (Thompson-Witrick et al., 2015). Moreover, it only requires a minimal amount of sample, which is important in the case of gluten-free breads that present a poor crust. Focusing on SPME-GC/MS volatile compounds

analyses, most researchers have studied the crumb and crust together (Paraskevopoulou, Chrysanthou, & Koutidou, 2012; Plessas et al., 2008, 2011; Poinot et al., 2007, 2008a). The study of the volatile compounds from the crust separately from the crumb is very important in order to understand its volatile profile. To our knowledge, only Raffo et al. (2015) and Pacyński et al. (2015) have studied the volatile compounds of bread crust by SPME-GC/MS, the latter examining gluten-free bread crust. On the other hand, understanding the performance characteristics of the analytical methodology is crucial in order to achieve reliable results, but this information has only been reported for SPME-GC/MS analyses of bread by Raffo et al. (2015). They studied the repeatability, intermediate precision, linearity as well as LOD and LOQ for volatile compounds analyses in wheat bread crust. However, to the best of our knowledge, the accuracy has not been studied for any SPME-GC/MS methodology; verifying the accuracy is very important for interpreting the quantifications made from these methodologies, since it expresses the closeness of the experimental result to the accepted value (AOAC guideline, 2002). Finally, the optimisation of the methodology before its validation is also imperative so as to ensure that the maximum amount of analyte is extracted, but any optimisation was carried out by Raffo et al. (2015) for the analysis of the volatile compounds of the crust by SPME-GC/QTOF. Moreover, as far as we know, the use of statistical tools such as the Response Surface Method (RSM) has not been reported for the optimisation of SPME methodologies for bread volatile compounds analyses.

Therefore, the first aim of this study was to optimise and validate a SPME-GC/quadrupole-time-of-flight (QTOF) methodology for the semi-quantification (lower limits of detection, since it works in splitless mode) and quantification (higher limits of detection, since it works in split mode) of 44 volatile compounds in bread crust, employing a commercial bread crust sample for this purpose. The quantification of the

commercial sample was made using the Method of Standard Addition (MSA). It must be noted that this is the first time that a SPME methodology has been optimised through the use of Design of Experiments (DOE) in the analysis of volatile compounds in bread, specifically with Principal Component Analysis (PCA) followed by RSM. The second goal was to quantify volatile compounds through the MSA of teff, basmati rice, japonica rice and wheat starch bread crusts for the selection of the most suitable gluten-free flour or starch for the improvement of the final aroma of gluten-free bread crust, using wheat bread as a control sample. The choice of the quantified gluten-free bread crusts was made using the semi-quantification method as screening process of oat, quinoa, teff, basmati rice, japonica rice and corn and wheat starch.

2. Materials and methods

2.1. Materials, reagents and standards

For the analytical characterisation of the method, 2-acetyl-1-pyrroline (2-ACPY) was purchased from Eptes (Vevey, Switzerland) and the other 43 pure standards found in Table S1 were purchased from Sigma-Aldrich (Steinheim, Germany). Dichloromethane was obtained from Scharlab (Barcelona, Spain) and methanol was from VWR International (Fontenay-sous-Bois, France). Argon, nitrogen and helium were acquired from Carburos Metálicos (Barcelona, Spain).

2.2. Preparation of standard solutions

2-ACPY solutions were prepared in dichloromethane, as 2-ACPY dimerises in methanol and water. It was necessary to work under inert atmosphere of argon at all times due to the compound's lack of stability to oxygen and moisture. For this reason, dichloromethane was dried in a SDS PS-MD-5 purification system from Düperthal Sicherheitstechnik (Karlstein am Main, Germany). For the other 43 volatile compounds

included in Table 1, working solutions of each volatile compound were prepared in methanol. All the solutions were stored in a freezer at -20°C.

2.3. Sample employed for the development of the SPME-GC/QTOF method

The development and characterisation of the methodology were carried out with the crust of wheat bread purchased from Forvasa (Puçol, Spain). The label indicated that the ingredients were wheat flour, water, salt, yeast and flour improver (wheat flour, anti-caking agent (E-170), emulsifier (E-472e), antioxidant (E-300) and enzymes).

Loaves of bread were cut into slices of 5 cm width, including the ends. The crust was scratched with a knife, taking care not to remove pieces of crumb. Once all the crust was removed, it was frozen with liquid nitrogen and finally it was grounded in an Ika grinder model M20 (Staufen, Germany) for 10 seconds.

2.4. Gluten-free bread formulation: flours, starches, hydrocolloid and yeast

Wheat starch was supplied by Roquette Laisa (Valencia, Spain), corn starch by Miwon Daesang (Seul, Korea) and wheat flour by Harinera Castellana (Medina del Campo, España). Japanica rice flour was purchased from Molendum ingredients (Zamora, Spain), oat flour from Emilio Esteban (Valladolid, Spain), quinoa flour from El Granero Integral (Madrid, Spain) and teff flour from Salutef (Palencia, Spain). Basmati flour was milled from basmati rice from Dacsa (Lisboa, Portugal), employing a grinder model Perten 3300 (Hägersten, Sweden). Hydroxyl propyl methyl cellulose (HPMC) K4M was supplied by Dow Chemicals (Michigan, USA) and the dry baker's yeast (*Saccharomyces cerevisiae*) by Lesaffre (Cerences, France). All yeasts belonged to the same batch to decrease the risk of different cell count of yeast and different contaminant bacteria.

2.5. Gluten-free bread making

The following ingredients, as g/100g of flour or starch, were used in all the formulas: sunflower oil (6 g/100 g), sucrose (5 g/100 g), salt (1.8 g/100g), yeast (3 g/100 g), HPMC (2 g/100 g) and water (100 g/100 g). They were mixed using a Kitchen-Aid Professional mixer (KPM5, KitchenAid, St. Joseph, Michigan, USA) for 8 min at speed of 56 rpm. The fermentation was carried out for 90 min in a chamber at 30°C with 90% of relative humidity, reaching average specific volumes between 1.91 ml/g and 6.89 ml/g (data not shown). The doughs were baked, in rows of two, at 190°C for 40 min in a convection oven model Salva 5 grid (Guipuzcoa, Spain). After baking, the gluten-free breads were left at room temperature for 30 min and cut as described in sub-section 2.3. Each sample was prepared in duplicate (n=2).

2.6. Solid-phase microextraction

Four fibres were tested, including polydimethysiloxane / divinylbenzene (PDMS/DVB) (65 µm), carboxen / polydimethysiloxane (CAR/PDMS) (85 µm), divinylbenzene / carboxen / polydimethysiloxane (DVB/CAR/PDMS) (50/30 µm) and polyacrylate (85 µm), all of them from Sigma Aldrich (Gillingham, UK). The selected fibre was DVB/CAR/PDMS and an autosampler was employed for the extraction of the volatile compounds. An amount of 0.75 g (± 0.0050 g) of wheat bread crust was weighed into a 20 mL vial and sealed with a magnetic screw cap provided with PTFE/silicone septa. The sample was incubated in the oven for 5 min at 60°C (without the fibre) and then the volatile compounds were extracted in the same oven for 51 min at 60°C, without agitation. After that, the fibre was inserted into the GC injector port for thermal desorption for 5 min at 270°C, with an injection volume of 1 µL. Finally, the fibre was conditioned for 30 min at 270°C after each analysis.

2.7. GC/QTOF chromatographic conditions

GC/QTOF analyses were performed on a 7890A gas chromatograph coupled to a 7200 Quadrupole-Time of flight (QTOF) mass spectrometer detector and MassHunter B.07.00 software, all from Agilent Technologies (Santa Clara, California, USA). The GC was equipped with a CombiPAL RSI 85 autosampler from CTC Analytics AG (Zwingen, Switzerland). The separation was achieved on a polar Innowax column (100% polyethylene glycol, 30 m × 0.25 mm ID × 0.25 μm) obtained from J&W Scientific (Agilent Technologies, California, USA). The chromatographic conditions were previously optimised by the research group using standard solutions (Pico, del Nozal, Bernal, & Gómez, 2017). The GC was operated under programmed temperature conditions: from 45°C (1.5 min) to 100°C (0 min) at 7°C/min, then the temperature was increased to 114°C (6.7 min) at 1°C/min, afterwards it was increased to 136°C (0 min) at 2.5°C/min and finally it was increased to 245°C (5 min) at 85°C/min. Total run time was 43 min. The carrier gas was helium at a flow rate of 1.1 mL/min. The injector temperature was 270°C, working in splitless mode for semi-quantitative analyses and in split mode for quantitative analyses. When the sample was spiked in the quantitative analysis using MSA, the most abundant compounds saturated the detector, thus it was compulsory to dilute the sample working in split mode. If the sample was spiked with less concentration, the increase in the signal was not sufficient to achieve good quantification. However, when the sample was not spiked there was no saturation and it was possible to work in splitless mode, increasing the sensitivity. The use of two working modes for different compounds was possible because the same volatile compound was studied in all the samples, which were injected in the same mode. However, different compounds injected in different modes were not compared. The interface, ion source and quadrupole temperatures were 250°C, 230°C and 150°C, respectively. Analyses were performed in SCAN mode and included a mass range of

20–350 m/z, operating in electron ionization mode with energy of 70 eV. All the 44 volatile compounds shown in Table S1 were identified by comparison of their retention times and accurate mass spectra (with four decimal places) with standards as well as using their Kovats Index (Table S1) and their Mass Spectra Library (NIST MS Search 2.2 & MS Interpreter).

2.8. Validation of the SPME-GC/QTOF method

The analytical parameters were evaluated following the AOAC guidelines (2002).

2.8.1. Limits of detection (LODs) and quantification (LOQs)

These parameters were calculated comparing the area of analyte peaks from a spiked crust sample and the area of the noise from a blank (the air of an empty vial) at the same retention time as that of the analyte peaks. Injections were made in quintuplicate (n=5). LODs were calculated as 3 times the signal to noise ratio (S/N), while LOQs were calculated as 10 times the S/N.

2.8.2. Precision: intra-day repeatability and inter-day repeatability

For intra-day repeatability, crust samples were injected in quintuplicate and the RSD (%) of each compound was calculated (n=5). In terms of inter-day repeatability, crust samples were injected in quintuplicate on three alternate days and RSD (%) was calculated (n=5). Following the AOAC guidelines (2002), maximum RSDs of 15% were accepted for the repeatability.

2.8.3. Quantification of volatile compounds of the commercial wheat bread crust sample: linearity and accuracy.

The quantification was made using the MSA. A matrix-matched calibration curve was made spiking six aliquots of the commercial crust sample with increasing concentration of the standard mixture (which contains the 44 volatile compounds) within the range of 0.150 – 1.30 mg Kg^{-1}. Six points were included in the calibration curve. The

coefficients of determination R^2 were obtained and a t-test for the linearity was done in order to ensure the linear tendency of the regression ($t_{experimental} > t_{critical}$). The relative errors (% Re) for the accuracy were also calculated. Following the AOAC guidelines (2002), maximum Re of 15% were accepted for accuracy. The concentration of the volatile compounds was calculated through MSA.

2.9. Application of the SPME-GC/QTOF method to the quantification of the volatile compounds of gluten-free bread crusts

The quantification was made in the same way than for the commercial bread, using the MSA. The volatile compounds of the crusts made with basmati rice, japonica rice and teff flours and wheat starch, as well as wheat flour (control sample), matrix-matched calibration curves were made in the range of 0.150 – 9.00 mg Kg^{-1} for all the volatile compounds except for 2-ACPY, which was from 0.0200 – 0.960 mg Kg^{-1}. The same procedure as that in sub-section 2.6 - 2.7 was followed.

2.10. Statistical analysis of the data.

The optimisation of the SPME methodology was made using a modification of a Central Composite Design (CCD) 3^3 (9 experiences), PCA and RSM. The modification of the CCD 3^3 as well as the RSM were computed by the software Statgraphics Centurion version XVII (Statpoint Technologies, Warrenton, Virginia), while PCA was done with the software LatentiX version 2.00 (Latent5, Copenhagen, Denmark), with data standardized prior to the analysis. In order to assess the variation of the volatile compounds among the different gluten-free bread crusts both in the semi-quantitative and quantitative studies, PCA were also conducted, which were the average of each bread crust sample prepared in duplicate and analysed in triplicate (n=6). In the case of the semi-quantitative studies, the peak areas were employed to perform the PCA; while

in the case of the quantitative studies, the concentrations in µg Kg^{-1} were the data employed for the PCA.

3. Results and discussion

3.1. Optimisation of the SPME conditions

3.1.1. Selection of the fibre

Four fibres were tested, including PDMS/DVB, CAR/PDMS, DVB/CAR/PDMS and polyacrylate. The conditions employed for the selection of the fibre were that of the set 1 of Table S2. All the fibres were cleaned at the temperature indicated by the manufacturer and blanks of air were run after each set of measurements in order to ensure the absence of interferences. Due to its high polarity, polyacrylate was the fibre that detected the fewest number of volatile compounds (Table S1) and important compounds like 2,3-butanedione, 1-methylpyrrol, heptanal or 2,3-dimethylpyrazine were not detected. Moreover, the peak areas of the remaining volatile compounds were the lowest of the four fibres. For PDMS/DVB, only 2-octanone was lacking and CAR/PDMS as well as DVB/CAR/PDMS detected the 44 volatile compounds. CAR/PDMS yielded the highest peak areas for the volatile compounds of low and medium molecular weights, such as hexanal, 1-methylpyrrol, acetoin, acetic acid, benzaldehyde or 2-methylbutanoic acid. Meanwhile, PDMS/DVB primarily showed the highest peak areas for the volatile compounds of high molecular weight, such as benzaldehyde, 2-(E)-nonenal, phenylacetaldehyde, benzyl alcohol or phenylethyl alcohol. DVB/CAR/PDMS presented the highest peak areas for 2,3-butanedione, 2-ethylpyrazine, 2-acetylpyrazine, 1-octen-3-ol and hexanoic acid and it yielded intermediate peak areas between PDMS/DVB and CAR/PDMS for the rest of the volatile compounds. Therefore, DVB/CAR/PDMS was selected as the best option for detection of volatiles compounds in gluten-free bread crusts.

3.1.2. Selection of the weight, extraction time and extraction temperature

In order to optimise the weight of sample, the extraction temperature and the extraction time, a modification of the Central Composite Design (CCD^3) was carried out (Table S2). The starting point of the design was the optimum (set 1, Table S2) obtained from a Central Composite Design (CCD^3) with three central points for the optimisation of a SPME-GC/QTOF methodology for the analysis of volatile compounds in flours and starches (Pico, Tapia, Bernal, & Gómez, 2017), since the moisture content of the flours and starches was not expected to be much higher than that of the crust (the moisture of our bread crusts ranged between 7.07% and 11.74%, data not shown). Nine combinations or sets of values of weight, extraction time and extraction temperature higher and lower than the optimum were used for experimentation (Table S2). The maximum value of weight (0.75 g) was selected in order to avoid the saturation of the detector, and the minimum (0.25 g) was selected due to the great losses of signal with lower weights. The maximum value of temperature (70 °C) was chosen so as to avoid the development of Maillard reactions, but with lower temperatures (50 °C) the signal decreased a lot. Finally, the minimum value of time (30 min) was selected due to the great losses of signal at lower times and the maximum time (75 min) because at higher times there was not visible increase of the signal.

PCA was employed in order to reduce the dimensionality of the original data (Ribeiro, Teófilo, Augusto, & Ferreira, 2010). Two principal components (PC1 and PC2) were sufficient to explain 86.2 % of the variance of the original variables. The RSM was then applied to the values of the two principal components of each volatile compound in the 9 experiments, as shown in Table S2. The optimum values of weight, extraction temperature and extraction time were 0.750 g, 60°C and 51 min, respectively. The "overall desirability function" (D), which estimates the suitability of the optimum for all

the volatile compounds at once (Bezerra, Santelli, Oliveira, Villar, & Escaleira, 2008), was 97.7 %.

3.2. Validation of the SPME-GC/QTOF method

3.2.1. Limits of detection (LODs) and quantification (LOQs)

For quantitative purposes, the method was performed in split 1:100 mode, as explained in subsection 2.7. The LODs ranged between 3.60 µg Kg^{-1} and 1.76 mg Kg^{-1} while the LOQs were between 12.0 µg Kg^{-1} and 5.85 mg Kg^{-1}, although the average values were 213 and 709 µg Kg^{-1}, respectively (Table 1).

For semi-quantitative purposes, the method was performed in splitless mode, with LODs ranging between 0.801 µg Kg^{-1} and 4.46 µg Kg^{-1} (Table 1). The LODs were much lower because the samples were not diluted.

3.2.2. Precision: intra-day repeatability and inter-day repeatability

Taking results from the quantitative method into consideration, as they allow for drawing better conclusions, the % RSD for intra-day repeatability varied between 0.120% and 7.89%, while values for the inter-day repeatability were between 0.830% and 9.00% (Table 1). Thus, the quantitative method was deemed sufficiently precise with no need for an internal standard.

3.2.3. Linearity and accuracy

For quantification of the commercial sample, the MSA was applied. Almost all the volatile compounds were verified by the t-test and almost all the R^2 were higher than 0.99 (data not shown), except for acetic acid and furfuryl alcohol. Both acetic acid and furfuryl alcohol presented R^2 values lower than 0.99, probably due to a lack of homogeneity in the blank (crust sample without spiking). The accuracy exhibited % Re between 0.085 and 12.2%, with an average value of 2.47% (Table 1). Therefore, the method was declared accurate.

3.3. Quantification of volatile compounds of commercial wheat bread crust

The results, in µg Kg^{-1}, of the concentration of the 44 volatile compounds quantified in the commercial bread crust sample are shown in Table 2. The most abundant compounds, in decreasing order, were furfural, butyrolactone, acetoin, phenylethyl alcohol, phenylacetaldehyde, 3-methyl-1-butanol, pyrazine, 1-hexanol, 2-methyl-1-propanol and 2-acetylpyrrol, with concentrations higher than 1 mg Kg^{-1}. Furfural has been also reported by Raffo et al. (2015) as the most abundant compound, by far, of the volatile compounds of wheat bread crust analysed by SPME-GC/MS. Phenylacetaldehyde, phenylethyl alcohol, 3-methyl-1-butanol, 1-hexanol and 2-acetylpyrrol were also reported by Raffo et al. (2015) and Pacyński et al. (2015), who also analysed the volatile compounds of wheat bread crust as well as gluten-free bread crust by SPME-GC/MS.

3-Methyl-1-butanol has been reported as the main volatile compound in wheat bread crumb generated by fermentation (Birch, Petersen, & Hansen, 2014), thus its presence in the crust should be due to a migration from the crumb. Phenylethyl alcohol and phenylacetaldehyde are mainly generated in crumb by fermentation, but they can also be generated in the crust by Strecker degradation during Maillard reactions (Birch et al., 2014; Pico et al., 2015). On the contrary, furfural is mainly generated in the crust by Maillard reactions (Jensen, Oestdal, Skibsted, Larsen, & Thybo, 2011; Martins, Jongen, & Van Boekel, 2000; Poinot et al., 2008b, 2010) and caramelisation from 5-hydroxymethyl-furfural (Ameur, Rega, Giampaoli, Trystram, & Birlouez-Aragon, 2008), although it can also be generated in the crumb during fermentation and then transferred to the crust (Birch et al., 2014; Pico et al., 2015). 1-Hexanol is mainly generated in the crumb by lipid oxidation and can be transferred to the crust. However, the hydroperoxides that bring about volatile compounds from lipid oxidation can also be

broken in the crust, generating volatile compounds too (Moskowitz et al., 2012; Pico et al., 2015). Therefore, 2-acetylpyrrol is the only one generated exclusively by Maillard reactions (Poinot et al., 2010) in the crust.

In accordance with Moskowitz et al. (2012), the main compounds found in our samples generated in the crust have been products of Maillard reactions and lipid oxidation reactions. 2-acetyl-pyrroline, 4-hydroxy-2,5-dimethyl-3(2H)-furanone as well as 2-acetylpyrazine have been reported as the major active volatile compounds from Maillard reactions in wheat bread crust (Moskowitz et al., 2012; Schieberle & Grosch, 1985; Zehentbauer & Grosch, 1998), and 2-(E)-nonenal and 2,4-(E,E)-decadienal have reportedly served as the major active volatile compounds from lipid oxidation reaction (Moskowitz et al., 2012). Consequently, 2-acetyl-1-pyrroline and 2-(E)-nonenal have been selected as the most important aroma contributors due to their high dilution factor (FD) (Schieberle & Grosch, 1991) and their low odour thresholds (OT), which are 0.053 µg Kg^{-1} and 0.08 µg Kg^{-1}, respectively, in water. Although they were not the most abundant compounds in our samples, all of these volatile compounds have been quantified in the commercial wheat bread crust of the present study.

3.4. Selection of the gluten-free flours or starches in order to quantify the volatile compounds of the corresponding bread crust: semi-quantitative analyses.

The volatile profile of five crusts of breads made with gluten-free flours (basmati rice, japonica rice, oat, teff and quinoa), two crusts of breads made with starches (corn and wheat) and the crust of wheat bread as a control sample, were studied in a semi-quantitative way in order to select the most suitable gluten-free flour or starch bread crust to be quantified (Table 3).

With the aim of reducing the number of volatile compounds of the data matrix and facilitating the interpretation of the results, only those volatile compounds with OTs

higher than 1 mg Kg^{-1} were considered. Then, the PCA was constructed with the 30 volatile compounds reported in Table S3, in peak areas, as shown in Figure 1. Moreover, in order to avoid that the highest areas had more importance in the weight of the PCs, the three first PCs were normalised as a correlation matrix (Table S3). Then, only those compounds with normalised PCs higher than 0.700 were taken into consideration in this discussion, which were calculated as the PC multiplied by the square root of the corresponding eigenvalue. These volatile compounds were: pyrazine, 2-methylpyrazine, 2,5,-dimethylpyrazine, 2,3,5-trimethylpyrazine, 2-ethyl-3-methylpyrazine, all from the Maillard reaction, and 2-(E)-nonenal, 2,4-(E,E)-decadienal and benzaldehyde, from lipid oxidation.

As an overview of the scores plot of the PCA (Figure 1), rice bread crust as well as teff bread crust were the samples with volatile profiles more similar to wheat bread crust. 4-Hydroxy-2,5-dimethyl-3(2H)-furanone, 4-vinylguaiacol, 2-(E)-nonenal and 2,4-(E,E)-decadienal were the volatile compounds that contributed more to the positive PC1 (Table S3); they were found in higher proportion in quinoa crust, wheat crust, teff crust and rice crust and in lower proportion in oat crust, basmati crust, corn starch crust and wheat starch crust. On the other hand, the negative PC1 was characterised by the highest contributions of pyrazines, including pyrazine, 2-methylpyrazine, 2,5-dimethylpyrazine, 2,3,5-trimethylpyrazine and 2-ethyl-3-methylpyrazine. Wheat starch crust presented the highest proportions of all of them, being expected that this would lead to a darker crust colour, but it was one of the lightest crusts (data not shown). Pyrazines have been reported as important Maillard compounds in bread crust (Paraskevopoulou, Chrysanthou, & Koutidou, 2012), that should contribute to its colour (Cho & Peterson, 2010); however, wheat flour crust was the darkest crust. This suggested that these pyrazines were not the responsible of the crust colour from compounds of Maillard

reactions. Additionally, furan derivatives have been reported to contribute to the colour of the heated food (Hofmann, 1998). Concretely, furfuryl alcohol has been reported to polymerise in acidic conditions to aliphatic polymers that give a brown colouration to the bread (Okaru & Lachenmeier, 2017). Wheat flour crust presented the highest abundance of furfuryl alcohol, which can explain it darkest colour.

Moreover, the similarity between wheat starch crust and wheat flour crust, most notably regarding the negative PC2, was related to the high content of pyrazines. Finally, the highest proportion of 2-ACPY was found in oat crust, although it was located opposite to wheat bread and its use was disregarded.

As a consequence, the crusts were distinguished mainly due to their content in pyrazines, 2-ACPY, 2-(E)-nonenal and 2,4-(E,E)-decadienal, as it was explained in sub-section 3.3. Therefore, since rice crust and teff crust were located near to wheat crust, they were selected to be quantified. Due to the high content of pyrazines, wheat starch crust was also selected to be quantified and basmati crust was chosen in order to study the effect of other varieties of rice.

3.5. Quantification of the volatile compounds of the selected gluten-free bread crusts: improvement of gluten-free bread crust aroma

Forty-two volatile compounds from wheat starch bread crust as well as teff, japonica rice and basmati rice bread crusts were quantified (Table 2). Wheat bread crust served as a control sample and acetic acid and furfuryl alcohol were excluded from the quantification, since their R^2 values were lower than 0.99 and they did not pass the t-test for linearity.

As for the semi-quantitative analysis, only those volatile compounds with OTs higher than 1 mg Kg^{-1} were taken into consideration. Then, the PCA was constructed with the volatile compounds labelled in Table S3 from number 1 to 28. The PCA of the

concentration of each volatile compound, in µg Kg^{-1}, is shown in Figure 2. Regarding the scores plot, basmati bread crust and wheat bread crust were located in the negative PC1 while wheat starch, rice and teff bread crusts were found in the positive PC1. In the negative PC1 of the loadings plot there were only 4 volatile compounds, which meant that both breads presented a less complex volatile profile in the crust. Basmatic crust was characterised by the highest content in 2-(E)-nonenal and limonene while wheat crust was characterised by the highest content in 4-hydroxy-2,5-dimethyl-3(2H)-furanone (similar to the content in wheat starch). The content of 2-(E)-nonenal in wheat crust was the second highest, almost 3 times higher than the third one (teff crust). Thus, the main difference between wheat crust and rice, teff and wheat starch crusts should be found in the content of 2-(E)-nonenal. There are some controversies about the impact of 2-(E)-nonenal on the final aroma of bread, since it has been reported as correlating positively with green notes (Hansen & Hansen, 1996; Salim-ur-Rehman, Paterson, & Piggott, 2006), but also negatively with fatty notes (Quílez, Ruiz, & Romero, 2006). In fact, it has been reported as one of the volatile compounds responsible for the staling of bread (Zehentbauer & Grosch, 1998). Regarding the negative notes generated during the staling of bread, the lower concentration of 2-(E)-nonenal in wheat starch, teff and rice crusts could be considered a positive attribute for gluten-free breads. Finally, although it contributed minimally to the correlation matrix (Table S3), 4-hydroxy-2,5-dimethyl-3(2H)-furanone from Maillard reactions (Moskowitz et al., 2012) has been reported as an important contributor to crust aroma (Zehentbauer & Grosch, 1998), with a caramel-like smell (Moskowitz et al., 2012).

In the positive PC1 of the loadings plot, 1-octen-3-ol, pyrazine, 2-methylpyrazine and 2,3,5-trimethylpyrazine were the volatile compounds in highest abundance; they were common for the rice, wheat starch and teff bread crusts. Moreover, all of them presented

correlation values higher than 0.75, thus they contributed to the overall flavour of the crust. 1-Octen-3-ol is a volatile compound from lipid oxidation that has been reported to correlate negatively with the final aroma of bread (Paraskevopoulou et al., 2012), presenting the highest concentration in the rice bread crust. Although the content of lipids is higher in teff than in rice and wheat starch (USDA Database, 2009), the amount of lipoxygenases is higher in rice (Wongdechsarekul & Kongkiattikajorn, 2010) and the concentration of antioxidants, such as flavonoids and vitamin E, is lower in rice (Inglett, Chen, & Liu, 2015). Then, the oxidation of lipids is encouraged in rice crust, justifying the highest amount of 1-octen-3-ol in rice crust (lower amount of lipids but higher lipoxygenase activity and lower antioxidant action) and in the second place in teff crust (higher amount of lipids but lower lipoxygenase activity and higher antioxidant action). In fact, rice crust and teff crust presented similar amounts of hexanal and nonanal, volatile compounds of lipid oxidation (Pico et al., 2015), which explained the balance between the content of lipids and the amount of lipoxygenases and antioxidants. The three pyrazines were in similar concentrations in rice, wheat starch and teff bread crusts, which could be one of the reasons for their separation from basmati and wheat crusts. 2,3,5-Trimethylpyrazine was the most abundant pyrazine overall and, in wheat starch crust, it was in highest concentration. In general, pyrazines have been reported as important Maillard compounds in bread crust (Paraskevopoulou et al., 2012), contributing greatly to its colour (Cho & Peterson, 2010). The darkest crust was that of teff, followed by rice and then wheat starch (data not shown), but the concentration of these pyrazines was not very different (see Table 2). This suggested, as in the semi-quantitative section, that pyrazine, 2-methylpyrazine and 2,3,5-trimethylpyrazine were not responsible for crust colour. The same reasoning could be applied to the highest content of 2,6-dimethylpyrazine in wheat starch crust.

Within the positive PC1, teff crust (negative component of the PC2) was separated from rice and wheat starch crust (positive component of the PC2). Higher contents of heptanal, 2,4-decadienal, 1-methylpyrrol and 2,5-dimethylpyrazine found in rice and wheat starch crusts compared to teff crust could explain this separation; meanwhile, teff was characterised by the highest content in fermentation volatile compounds like acetoin, phenylacetaldehyde and 3-methylbutanoic acid. However, these three fermentation volatile compounds did not show high values of correlation (Table S3), probably because their presence depended on the migration from the crumb to the crust and not on their homogeneous generation in the crust. The same occurred with the highest content of 3-methyl-1-butanol in wheat starch crust, which had a content similar to that of wheat flour crust. Heptanal and 2,4-decadienal, with correlation values higher than 0.70, are lipid oxidation volatile compounds (Birch et al., 2014) with the highest concentration in rice, probably due to the same reasons explained for 1-octen-3-ol. 1-Methylpyrrol as well as 2,5-dimethylpyrazine, both volatile compounds from the Maillard reaction (Poinot et al., 2008b), had high values of correlation, although they have not been reported as important contributors to the crust aroma.

Therefore, the gluten-free bread crusts were mainly distinguished by their contents of volatile compounds from lipid oxidation and Maillard reactions, which have been reported as the main compounds in the crust of wheat bread (Moskowitz et al., 2012), as it was explained in sub-section 3.4. For wheat bread crust, the control sample, the most abundant volatile compound was 4-hydroxy-2,5-dimethyl-3(2H)-furanone (26.8 µg Kg^{-1}). This could be the reason for the similarity between wheat flour crust and teff and wheat starch crusts, as the contents of 4-hydroxy-2,5-dimethyl-3(2H)-furanone were 20.0 µg Kg^{-1} and 22.6 µg Kg^{-1}, respectively. Moreover, the contents of the key aroma 2-ACPY in wheat flour bread crust and in teff and wheat starch bread crusts were also

similar (0.0459 µg Kg^{-1}, 0.0398 µg Kg^{-1} and 0.0321 µg Kg^{-1}, respectively), explaining the likeness of teff and wheat starch crusts regarding wheat flour crust. In the case of teff crust, 2-ACPY was the most abundant compound followed by 2-ethyl-3-methylpyrazine (32.1 µg Kg^{-1}), while in the case of wheat starch crust 2,3,5-trimethylpyrazine was the most abundant (48.1 µg Kg^{-1}) and 2-ACPY was second in abundance. Therefore, a suitable mixture between wheat starch and teff flour was suggested in order to improve the final aroma of gluten-free bread.

By contrast, Pacyński et al. (2015) reported that their gluten-free breads were characterised by a lack of pyrazines and 2-acetyl-1-pyrroline compared to the control wheat bread. However, we found contents of pyrazines that varied between 0.440 and 48.1 µg Kg^{-1} among the four gluten-free breads and contents of 2-ACPY that varied between 0.210 and 39.8 µg Kg^{-1}. The differences are surprising since Pacyński et al. (2015) added, besides corn and wheat starches, sources of amino acids and sugars that encourage the Maillard reaction, like glucose, milk powder and egg. Therefore, it would be expected that pyrazines and other compounds from Maillard reaction were found in the gluten-free breads studied by Pacyński et al. (2015).

4. Conclusions

A SPME-GC/QTOF methodology for the analyses of 44 volatile compounds in bread crusts has been developed, optimised and validated. The optimisation was accomplished with the application of the Response Surface Method (RSM), with previous reduction of the dimensionality employing Principal Component Analysis (PCA). The final SPME conditions were 0.75 g of crust extracted at 60°C for 51 min. The SPME-GC/QTOF methodology was validated in terms of LOD and LOQ, precision, accuracy and linearity, proving that it was sensible, precise, accurate and linear. The methodology was applied to quantification through the Method of Standard Addition (MSA) of a

commercial wheat bread crust. Furfural, which comes from Maillard reactions and caramelisation processes, was the most abundant compound in the commercial wheat flour bread crust, corresponding with the literature. Four selected gluten-free bread crusts (rice, basmati, teff and wheat starch) were also quantified and compared with a wheat bread crust control sample. It was concluded that wheat starch crust as well as teff crust were the closest to the control wheat crust due to their similar contents in 2-acetyl-1-pyrroline (2-ACPY), 4-hydroxy-2,5-dimethyl-3(2H)-furanone and pyrazines, which have been reported as main compounds in wheat bread crust.

Acknowledgements

Joana Pico and Laura Román would like to thank the University of Valladolid for her PhD fellowships. The authors acknowledge the financial support of the Spanish Ministry of Economy and Competitiveness (Project AGL2014-52928-C2-2-R) and the European Regional Development Fund (FEDER). The authors would like to thank Anna Hayes for the revision of the English scientific language. Finally, the authors want also to thank Eptes (Vevey, Switzerland) for their kindness when we purchased the 2-acetyl-1-pyrroline standard.

References

AOAC guideline (2002). *Last access 5th December 2017* https://www.aoac.org/aoac_prod_imis/AOAC_Docs/StandardsDevelopment/SLV_Guidelines_Dietary_Supplements.pdf

Ameur, L. A., Rega, B., Giampaoli,P., Trystram, G., Birlouez-Aragon, I. (2008). The fate of furfurals and other volatile markers duringthe baking process of a model cookie. *Food Chemistry*, *111*, 758-753.

Bezerra, M. A., Santelli, R. E., Oliveira, E. P., Villar, L. S., & Escaleira, L. A. (2008). Response surface methodology (RSM) as a tool for optimization in analytical chemistry. *Talanta*, *76*(5), 965–977.

Birch, A. N., Petersen, M. A., & Hansen, Å. S. (2014). Aroma of Wheat Bread Crumb. *Cereal Chemistry*, *91 (2)*, 105–114.

Cho, I. H., & Peterson, D. G. (2010). Chemistry of bread aroma: A review. *Food Science and Biotechnology*, *19*(3), 575–582.

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

Inglett, G. E., Chen, D., & Liu, S. X. (2015). Antioxidant Activities of Selective Gluten Free Ancient Grains. *Food and Nutrition* Science, *6*, 612–621.

Jensen, S., Oestdal, H., Skibsted, L. H., Larsen, E., & Thybo, A. K. (2011). Chemical changes in wheat pan bread during storage and how it affects the sensory perception of aroma, flavour, and taste. *Journal of Cereal Science*, *53*(2), 259–268.

Martins, S. I. F. S., Jongen, W. M. F., & Van Boekel, M. A. J. S. (2000). A review of Maillard reaction in food and implications to kinetic modelling. *Trends in Food Science and Technology*, *11*, 364–373.

Moskowitz, M. R., Bin, Q., Elias, R. J., & Peterson, D. G. (2012). Influence of endogenous ferulic acid in whole wheat flour on bread crust aroma. *Journal of Agricultural and Food Chemistry*, *60*, 11245–11252.

Pacyński, M., Wojtasiak, R. Z., & Mildner-Szkudlarz, S. (2015). Improving the aroma of gluten-free bread. *LWT - Food Science and Technology*, *63*(1), 706–713.

Paraskevopoulou, A., Chrysanthou, A., & Koutidou, M. (2012). Characterisation of volatile compounds of lupin protein isolate-enriched wheat flour bread. *Food Research International*, *48*(2), 568–577.

Pico, J., Bernal, J., & Gómez, M. (2015). Wheat bread aroma compounds in crumb and crust: A review. *Food Research International*, *75*, 200–215.

Pico, J., del Nozal, M.J., Bernal, J., & Gómez, M. (2017). Inhibition of fermentation evolution in bread doughs for aroma analyses. *Flavour and Fragance Journal*, DOI: 10.1002/ffj.3405.

Pico, J., Tapia, J., Bernal, J., & Gómez, M. (2017). Comparison of different extraction methodologies for the analysis of volatile compounds in gluten-free flours and corn starch by GC/QTOF. *Food Chemistry*, http://dx.doi.org/10.1016/j.foodchem.2017.06.157.

Plessas, S., Alexopoulos, A., Bekatorou, A., Mantzourani, I., Koutinas, A. A., & Bezirtzoglou, E. (2011). Examination of freshness degradation of sourdough bread made with kefir through monitoring the aroma volatile composition during storage. *Food Chemistry*, *124*(2), 627–633.

Plessas, S., Fisher, A., Koureta, K., Psarianos, C., Nigam, P., & Koutinas, A. A. (2008). Application of Kluyveromyces marxianus, Lactobacillus delbrueckii ssp. bulgaricus and L. helveticus for sourdough bread making. *Food Chemistry*, *106*, 985–990.

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.

Poinot, P., Arvisenet, G., Grua-Priol, J., Fillonneau, C., Le-Bail, A., & Prost, C. (2010). Influence of inulin on bread: Kinetics and physico-chemical indicators of the formation of volatile compounds during baking. *Food Chemistry*, *119*(4), 1474–1484.

Poinot, P., Grua-Priol, J., Arvisenet, G., Rannou, C., Semenou, M., Bail, A. Le, & Prost, C. (2007). Optimisation of HS-SPME to study representativeness of partially baked bread odorant extracts. *Food Research International*, *40*, 1170–1184.

Quílez, J., Ruiz, J. A., & Romero, M. P. (2006). Relationships between sensory flavor evaluation and volatile and nonvolatile compounds in commercial wheat bread type baguette. *Journal of Food Science*, *71*.

Raffo, A., Carcea, M., Castagna, C., & Magrì, A. (2015). Improvement of a headspace solid phase microextraction-gas chromatography/mass spectrometry method for the analysis of wheat bread volatile compounds. *Journal of Chromatography A*, *1406*, 266–278.

Ribeiro, J. S., Teófilo, R. F., Augusto, F., & Ferreira, M. M. C. (2010). Simultaneous optimization of the microextraction of coffee volatiles using response surface methodology and principal component analysis. *Chemometrics and Intelligent Laboratory Systems*, *102*(1), 45–52.

Salim-ur-Rehman, Paterson, A., & Piggott, J. R. (2006). Flavour in sourdough breads: a review. *Trends in Food Science and Technology*, *17*, 557–566.

Schieberle, P., & Grosch, W. (1985). Identification of the volatile flavour compounds of wheat bread crust - comparison with rye bread crust. *Zeitschrift Für Lebensmittel-Untersuchung Und -Forschung*, *180*, 474–478.

Schieberle, P., & Grosch, W. (1991). Potent odorants of the wheat bread crumb Differences to the crust and effect of a longer dough fermentation. *Zeitschrift Für Lebensmittel-Untersuchung Und -Forschung*, *192*, 130–135.

USDA Database: https://ndb.nal.usda.gov/ndb/ (*Last Access 19nd September 2017)*

Wongdechsarekul, S., & Kongkiattikajorn, J. (2010). Storage time affects storage proteins and volatile compounds , and pasting behavior of milled rice. *KKU Research Journal*, *15*(9), 852–862.

Zehentbauer, G., & Grosch, W. (1998). Crust Aroma of Baguettes I. Key Odorants of Baguettes Prepared in Two Different Ways. *Journal of Cereal Science*, *28*, 81–92.

Table 1. LODs and LOQs (in µg Kg^{-1}), repeatability (%RSD) and accuracy (%Re) of the 44 studied volatile compounds with proposed quantitative method. The LODs of the qualitative method are also given. The numeration given in parentheses of each volatile compound corresponds to the number assigned in the corresponding PCA (Figures 1 and 2).

Volatile compound	LOD split 1:100	LOQ split 1:100	LOD splitless	% RSD Intraday	% RSD Interday	% Re
2,3-Butanedione **(1)**	131	438	0.801	2.24	4.60	3.33
Hexanal **(2)**	62.8	209	2.53	2.39	4.40	1.82
2-Methyl-1-propanol	57.6	192	1.38	0.480	4.60	3.59
1-Methylpyrrol **(3)**	28.6	95.2	1.49	0.400	0.800	3.15
Heptanal **(4)**	101	338	1.27	1.65	1.50	3.18
R-Limonene **(5)**	5.70	19.0	2.54	0.780	6.50	4.14
Pyrazine **(6)**	54.2	181	3.42	0.750	5.60	1.47
2-Methyl-1-butanol	128	427	0.77	5.75	4.80	2.12
3-Methyl-1-butanol **(7)**	140	467	0.78	3.96	5.10	0.977
1-Pentanol	402	1340	1.00	6.23	9.00	1.57
2-Methylpyrazine **(8)**	53.6	179	0.15	0.530	1.30	0.324
Acetoin **(9)**	476	1586	1.49	2.73	2.90	0.821
2-Octanone **(10)**	40.2	134	0.34	4.52	4.70	10.9
2,5-Dimethylpyrazine **(11)**	22.7	75.5	0.18	4.95	3.70	3.27
2,6-Dimethylpirazine **(12)**	35.0	117	1.39	3.73	4.90	1.93
2-Ethylpyrazine	28.6	95.5	0.24	0.670	2.30	0.847
2-Acetyl-1-pyrroline **(13)**	12.1	39.9	0.19	1.25	3.21	0.981
2,3-Dimethylpyrazine	25.6	85.3	2.53	0.500	4.10	2.57
1-Hexanol	105	349	0.81	0.760	2.10	1.55
Nonanal **(14)**	102	341	2.47	2.57	3.70	0.235
2,3,5-trimethylpyrazine **(15)**	4.70	15.6	1.76	0.570	4.50	1.31
2-Ethyl-3-methylpirazine **(16)**	8.00	26.6	1.90	0.930	4.30	2.34
Ethyl octanoate **(17)**	3.60	12.0	0.65	5.39	8.60	2.55
1-Octen-3-ol **(18)**	8.40	27.9	3.67	3.58	3.50	0.281
Acetic acid **(29)**	49.3	164	1.62	7.03	1.00	nq

Table 1. (continued)

Volatile compound	LOD split 1:100	LOQ split 1:100	LOD splitless	% RSD Intraday	% RSD Interday	% Relative error
Furfural	50.3	168	3.61	2.38	2.70	7.02
2-Ethyl-1-hexanol **(19)**	22.6	75.4	2.01	5.17	4.80	0.648
Benzaldehyde **(20)**	19.7	65.7	1.84	0.830	4.10	0.085
2-(E)-Nonenal **(21)**	43.6	146	1.91	1.13	3.10	12.2
5-Methyl-2-furaldehyde	87.7	292	0.21	0.130	2.40	3.94
Butyrolactone	743	2477	1.38	4.42	7.40	5.14
2-Acetilpyrazine **(22)**	15.3	51.0	0.95	1.27	4.90	2.56
Butyric acid **(23)**	392	1307	0.81	1.31	2.50	1.58
Phenylacetaldehyde**(24)**	28.50	94.8	0.68	1.32	2.20	0.683
Furfuryl alcohol **(30)**	66.6	222	1.93	1.84	4.10	nq
2-Methylbutyric acid	225	751	4.46	5.51	1.20	2.58
3-Methylbutyric acid **(25)**	667	2224	3.17	7.89	1.50	0.229
2,4-(E,E)-Decadienal **(26)**	25.9	86.3	1.73	5.20	4.30	3.05
Hexanoic acid	1540	5132	0.85	0.120	2.40	0.374
Benzyl alcohol	67.8	226	0.77	0.560	7.20	1.66
Phenylethyl alcohol	48.8	163	2.76	3.91	8.60	0.425
2-Acetylpyrrol	290	966	1.34	3.86	4.90	1.68
4-Hydroxy-2,5-dimethyl-3(2H)-furanone **(27)**	1755	5851	3.61	3.74	2.70	0.945
4-Vinylguaiacol **(28)**	985	3284	3.15	3.73	5.20	1.83

* nq = not quantified

Table 2. Concentration, in μg Kg^{-1}, of the 44 studied volatile compounds in the crusts of wheat, basmati rice, japonica rice, wheat starch and teff breads as well as in the commercial bread. Values are means of three determinations ± SD. Different letters in the same row show the significant differences, excluding the commercial crust.

Volatile compound	**μg Kg^{-1} wheat flour crust**	**μg Kg^{-1} basmati rice crust**	**μg Kg^{-1} japonica rice crust**	**μg Kg^{-1} wheat starch crust**	**μg Kg^{-1} teff crust**	**μg Kg^{-1} commercial crust**
2,3-Butanedione	4.98 a ± 0.112	5.21 a ± 0.117	44.3 d ± 0.992	13.0 b ± 0.291	19.1 c ± 0.427	0.824 ± 0.427
Hexanal	4.36 b ± 0.104	7.06 c ± 0.169	16.7 d ± 0.400	1.69 a ± 0.0404	17.1 d ± 0.409	0.907 ± 0.409
2-Methyl-1-propanol	8.93 b ± 0.0429	14.3 c ± 0.0686	71.4 e ± 0.343	1.09 a ± 0.00523	17.8 d ± 0.0852	1.04 ± 0.0852
1-Methylpyrrol	1.40 b ± 0.00560	0.0700 a ± 0.000280	2.10 d ± 0.00840	2.13 d ± 0.00852	1.62 c ± 0.00648	0.386 ± 0.00648
Heptanal	14.2 c ± 0.235	0.500 a ± 0.00825	20.6 e ± 0.340	16.9 d ± 0.279	6.06 b ± 0.100	0.903 ± 0.100
R-Limonene	0.0100 a ±0.0000780	11.5 e ± 0.0895	0.220 b ± 0.00172	1.49 c ± 0.0116	1.90 d ± 0.0148	0.118 ± 0.0148
Pyrazine	4.21 b ± 0.0316	0.920 a ± 0.00690	7.70 d ± 0.0578	6.75 c ± 0.0506	9.77 e ± 0.0733	1.11 ± 0.0733
2-Methyl-1-butanol	7.49 c ± 0.431	3.32 a ± 0.191	5.29 b ± 0.304	4.81 ab ± 0.277	12.4 d ± 0.714	0.676 ± 0.714
3-Methyl-1-butanol	10.5 c ± 0.417	8.63 b ± 0.342	10.7 c ± 0.424	11.1 c ± 0.438	6.94 a ± 0.275	1.56 ± 0.275
1-Pentanol	1.88 a ± 0.117	6.77 c ± 0.422	4.90 b ± 0.305	4.64 b ± 0.289	6.64 c ± 0.414	0.609 ± 0.414
2-Methylpyrazine	14.0 d ± 0.0741	1.57 a ± 0.00832	19.8 e ± 0.105	11.5 b ± 0.0612	12.4 c ± 0.0657	0.871 ± 0.0657
Acetoin	9.36 c ± 0.256	4.30 a ± 0.117	7.71 b ± 0.210	9.38 c ± 0.256	27.8 d ± 0.758	4.34 ± 0.758
2-Octanone	21.8 d ± 0.983	0.640 a ± 0.0289	14.0 c ± 0.633	10.8 b ± 0.488	10.2 b ± 0.461	0.000100 ± 0.461
2,5-Dimethylpyrazine	10.7 b ± 0.532	0.820 a ± 0.0406	38.1 d ± 1.89	23.2 c ± 1.15	0.790 a ± 0.0391	0.143 ± 0.0391
2,6-Dimethylpirazine	4.70 c ± 0.175	0.760 a ± 0.0283	0.150 a ± 0.00560	11.6 d ± 0.431	2.01 b ± 0.0750	0.237 ± 0.0750

Table 2. (continued)

Volatile compound	µg Kg^{-1} wheat flour crust	µg Kg^{-1} basmati rice crust	µg Kg^{-1} japonica rice crust	µg Kg^{-1} wheat starch crust	µg Kg^{-1} teff crust	µg Kg^{-1} commercial crust
2-Ethylpyrazine	6.52 d ± 0.0437	0.860 c ± 0.00576	0.330 a ± 0.00221	8.09 e ± 0.0542	0.640 b ± 0.00429	0.358 ± 0.00429
2-Acetyl-1-pyrroline	0.0459 e ± 0.000574	0.000210 a ± 0.00000263	0.00743 b ± 0.0000928	0.0321 c ± 0.000402	0.0398 d ± 0.000498	0.0146 ±0.000221
2,3-Dimethylpyrazine	14.9 e ± 0.0744	0.820 a ± 0.00410	5.75 b ± 0.0288	11.3 d ± 0.0564	8.47 c ± 0.04235	0.255 ± 0.04235
1-Hexanol	15.4 c ± 0.117	0.320 a ± 0.00243	0.770 b ± 0.00585	0.700 b ± 0.00532	21.4 d ± 0.163	1.11 ± 0.163
Nonanal	2.95 b ± 0.0758	0.640 a ± 0.0164	4.13 c ± 0.106	2.75 b ± 0.0707	4.21 c ± 0.108	0.0598 ± 0.108
2,3,5-trimethylpyrazine	0.730 a ± 0.00416	0.440 a ± 0.00251	37.1 c ± 0.212	48.1 d ± 0.274	28.9 b ±0.165	0.0661 ± 0.165
2-Ethyl-3-methylpirazine	0.420 a ± 0.00391	0.600 a ± 0.00558	26.8 b ± 0.249	0.460 a ± 0.00428	32.1 c ± 0.298	0.127 ± 0.298
Ethyl octanoate	0.240 a ± 0.0129	0.0900 a ± 0.00485	3.83 c ± 0.206	0.690 b ± 0.0372	0.150 a ± 0.00809	0.360 ± 0.00809
1-Octen-3-ol	4.48 b ± 0.160	0.600 a ± 0.0215	24.1 d ± 0.862	16.5 c ± 0.591	18.5 c ± 0.663	0.338 ± 0.663
Acetic acid	nq*	nq*	nq*	nq*	nq*	nq*
Furfural	7.35 c ± 0.175	4.49 b ± 0.107	0.760 a ± 0.0181	26.1 d ± 0.622	1.10 a ± 0.0262	7.68 ± 0.0262
2-Ethyl-1-hexanol	0.290 a ± 0.0150	0.440 a ± 0.0227	0.250 a ± 0.0129	17.7 b ± 0.913	0.740 a ± 0.0383	0.139 ± 0.0383
Benzaldehyde	0.210 a ± 0.00174	0.340 a ± 0.00282	15.8 b ± 0.131	0.380 a ± 0.00315	0.320 a ± 0.00266	0.116 ± 0.00266
2-(E)-Nonenal	0.970 d ± 0.0110	4.43 e ± 0.0501	0.210 b ± 0.00237	0.0400 a ± 0.000452	0.370 c ± 0.00418	0.0329 ± 0.00418
5-Methyl-2-furaldehyde	0.450 d ± 0.000585	0.0900 a ± 0.000117	0.150 b ± 0.000195	0.220 c ± 0.000286	0.500 e ± 0.000650	0.0357 ± 0.000650

Table 2. (continued)

Butyrolactone	9.39 c ± 0.415	17.2 d ± 0.762	6.71 ab ± 0.297	8.08 bc ± 0.357	6.15 a ± 0.272	6.48 ± 0.272
2-Acetilpyrazine	12.0 c ± 0.152	0.0600 a ± 0.000762	4.97 b ± 0.0631	0.110 a ± 0.00140	0.130 a ± 0.00165	0.0799 ± 0.00165
Butyric acid	5.28 c ± 0.0692	0.170 a ± 0.00223	8.08 e ± 0.106	2.17 b ± 0.0284	6.55 d ± 0.0858	0.283 ± 0.0858
Phenylacetaldehyde	4.70 c ± 0.0620	2.50 b ± 0.0330	0.610 a ± 0.00805	1.08 a ± 0.0143	22.1 d ± 0.292	1.83 ± 0.292
Furfuryl alcohol	nq*	nq*	nq*	nq*	nq*	nq*
2-Methylbutyric acid	3.45 b ± 0.190	1.64 a ± 0.0904	9.21 d ± 0.507	3.97 b ± 0.219	6.35 c ± 0.350	0.176 ± 0.350
3-Methylbutyric acid	4.74 b ± 0.374	2.41 a ± 0.190	5.49 b ± 0.433	9.32 c ± 0.735	12.6 d ± 0.996	0.397 ± 0.996
2,4-(E,E)-Decadienal	0.110 a ± 0.00572	0.350 b ± 0.0182	1.13 d ± 0.0588	0.630 c ± 0.0328	0.450 b ± 0.0234	0.0247 ± 0.0234
Hexanoic acid	13.0 c ± 0.0156	18.3 e ± 0.0219	15.0 d ± 0.0180	2.54 a ± 0.00305	10.8 b ± 0.0130	0.0246 ± 0.0130
Benzyl alcohol	0.290 a ± 0.00162	0.160 a ± 0.000896	34.4 b ± 0.193	0.220 a ± 0.00123	0.110 a ± 0.000616	0.104 ± 0.000616
Phenylethyl alcohol	13.0 b ± 0.510	5.54 a ± 0.217	56.5 d ± 2.21	2.02 a ± 0.0790	18.6 c ± 0.726	3.59 ± 0.726
2-Acetylpyrrol	4.38 b ± 0.169	0.450 a ± 0.0174	0.300 a ± 0.0116	27.6 c ± 1.06	5.97 b ± 0.230	1.02 ± 0.230
4-Hydroxy-2,5-dimethyl-3(2H)-furanone	26.8 e ± 1.00	7.69 b ± 0.288	3.96 a ± 0.148	22.6 d ± 0.845	20.0 c ± 0.746	0.572 ± 0.746
4-Vinylguaiacol	1.93 a ± 0.0720	13.0 c ± 0.486	4.16 b ± 0.155	2.39 a ± 0.0891	22.8 d ± 0.851	0.758 ± 0.851

*nq = not quantified

Table 3. Peak areas, divided by 10^6, of the 44 studied volatile compounds in the crusts of corn starch, wheat starch, basmati, rice, oat, teff, quinoa and wheat breads as well as in the commercial bread. Values are means of three determinations ± SD. Different letters in the same row show the significant differences.

Volatile compound	Corn starch crust	Wheat starch crust	Basmati rice crust	Japonica rice crust	Oat crust	Teff Crust	Quinoa crust	Wheat flour crust
2,3-Butanedione	0.876 ab ± 0.0449	0.826 ab ± 0.0991	1.11 b ± 0.0983	0.991 b ± 0.0987	0.605 a ± 0.0710	0.919 ab ± 0.231	0.960 b ± 0.251	1.05 b ± 0.138
Hexanal	3.78 a ± 0.112	6.31 e ± 0.535	4.76 b ± 0.362	7.46 f ± 0.408	5.84 de ± 0.294	5.37 bcd ± 0.200	5.66 cde ± 0.140	5.11 bc ± 0.0208
2-Methyl-1-propanol	0.100 ab ± 0.0115	0.190 d ± 0.00213	0.183 cd ± 0.0209	0.252 e ± 0.0346	0.0613 a ± 0.00648	0.252 e ± 0.0375	0.0611 a ± 0.00857	0.141 bc ± 0.00215
1-Methylpyrrol	0.0374 a ± 0.00513	0.116 c ± 0.0123	0.102 c ± 0.00139	0.0658 b ± 0.00300	0.0476 ab ± 0.00512	0.160 d ± 0.0208	0.124 c ± 0.0139	0.116 c ± 0.00460
Heptanal	0.949 a ± 0.0531	1.41 bc ± 0.0771	1.08 ab ± 0.0489	2.22 d ± 0.0214	1.23 ab ± 0.0316	3.17 f ± 0.178	2.65 e ± 0.124	1.74 c ± 0.355
R-Limonene	0.416 a ± 0.0570	2.82c ± 0.214	1.88 b ± 0.311	2.32 c ± 0.314	0.773 a ± 0.216	1.88 b ± 0.232	2.54 c ± 0.334	2.36 c ± 0.291
Pyrazine	2.62 bc ± 0.0211	3.60 d ± 0.132	3.59 d ± 0.296	3.02 c ± 0.115	2.26 b ± 0.216	2.38 b ± 0.284	0.636 a ± 0.0489	3.01 c ± 0.0482
2-Methyl-1-butanol	1.38 a ± 0.218	3.30 e ± 0.186	2.53 cd ± 0.191	2.84 d ± 0.295	1.01 a ± 0.0368	2.06 b ± 0.224	1.04 a ± 0.0546	2.19 bc ± 0.218

Table 3. (continued)

Volatile compound	Corn starch crust	Wheat starch crust	Basmati rice crust	Japonica rice crust	Oat crust	Teff Crust	Quinoa crust	Wheat flour crust
3-Methyl-1-butanol	2.63 b ± 0.254	3.89 d ± 0.342	3.81 cd ± 0.0323	3.75 cd ± 0.0501	2.28 ab ± 0.113	3.50 c ± 0.0440	2.16 a ± 0.000333	3.65 cd ± 0.132
1-Pentanol	0.216 a ± 0.0319	0.793 bc ± 0.258	0.799 bc ± 0.0745	1.80 d ± 0.305	1.07 c ± 0.0309	0.731 b ± 0.0450	1.07 c ± 0.0225	0.817 bc ± 0.00182
2-Methylpyrazine	0.856 c ± 0.00643	2.14 f ± 0.00514	1.26 e ± 0.0352	1.09 d ± 0.00524	1.22 e ± 0.00658	1.15 d ± 0.0351	0.158 a ± 0.00877	0.773 b ± 0.0589
Acetoin	4.43 ab ± 0.0937	4.18 a ± 0.172	5.19 c ± 0.0738	4.64 b ± 0.0573	5.74 d ± 0.231	4.61 b ± 0.0342	6.32 e ± 0.176	5.97 de ± 0.291
2-Octanone	0.542 ab ± 0.0680	1.42 e ± 0.0662	0.629 c ± 0.0212	0.894 d ± 0.0256	0.366 a ± 0.00951	1.05 d ± 0.150	1.33 e ± 0.137	1.02 d ± 0.140
2,5-Dimethylpyrazine	0.185 b ± 0.0118	0.715 f ± 0.0414	0.338 d ± 0.00568	0.289 c ± 0.0211	0.667 e ± 0.00713	0.358 d ± 0.0166	0.0432 a ± 0.00512	0.179 b ± 0.0199
2,6-Dimethylpirazine	0.190 b ± 0.0154	0.299 d ± 0.00149	0.186 b ± 0.00286	0.184 b ± 0.00158	0.416 e ± 0.0129	0.199 bc ± 0.00468	0.0622 a ± 0.00162	0.221 c ± 0.138
2-Ethylpyrazine	0.0941 b ± 0.00348	0.258 f ± 0.00484	0.157 d ± 0.00361	0.152 d ± 0.00768	0.157 d ± 0.00767	0.210 e ± 0.00940	0.0442 a ± 0.00424	0.129 c ± 0.0208

Table 3. (continued)

Volatile compound	Corn starch crust	Wheat starch crust	Basmati rice crust	Japonica rice crust	Oat crust	Teff Crust	Quinoa crust	Wheat flour crust
2-Acetyl-1-pyrroline	0.772 c ± 0.0411	1.07 d± 0.0759	0.690 bc ± 0.105	0.318 a ± 0.00103	1.37 e ± 0.0309	0.366 a ± 0.0878	0.498 ab ± 0.0838	0.317 a ± 0.0518
2,3-Dimethylpyrazine	3.55 b ± 0.00348	5.81 e ± 0.0737	3.65 b ± 0.262	3.48 b ± 0.0415	5.13 d ± 0.0641	3.53 b ± 0.215	0.817 a ± 0.0294	4.18 c ± 0.00460
1-Hexanol	0.591 a ± 0.0934	1.46 c ± 0.212	1.29 c ± 0.129	2.81 d ± 0.106	0.918 b ± 0.0738	3.66 e ± 0.203	3.95 e ± 0.0109	1.53 c ± 0.355
Nonanal	1.64 a ± 0.296	1.57 a ± 0.691	1.31 a ± 0.281	1.83 a ± 0.401	1.80 a ± 0.239	1.72 a ± 0.497	1.93 a ± 0.401	1.75 a ± 0.291
2,3,5-trimethylpyrazine	0.111 b ± 0.00363	0.567 f ± 0.0370	0.162 c ± 0.0150	0.144 bc ± 0.00613	0.333 e ± 0.00146	0.200 d ± 0.000373	0.0445 a ± 0.00381	0.127 b ± 0.0482
2-Ethyl-3-methylpirazine	1.84 b ± 0.0624	4.90 g ± 0.0361	2.20 c ± 0.102	2.44 cd ± 0.187	3.48 e ± 0.242	3.80 f ± 0.128	0.777 a ± 0.0109	2.61 d ± 0.218
Ethyl octanoate	2.88 ab ± 0.458	4.40 cd ± 0.485	3.34 bc ± 0.517	3.12 ab ± 0.482	1.97 a ± 0.235	4.75 d ± 0.317	4.05 bcd ± 0.840	3.32 bc ± 0.132
1-Octen-3-ol	0.0523 c ±0.0000341	0.0293 ab ± 0.00329	0.0319 b ± 0.00522	0.0323 b ± 0.00702	0.0214 a ± 0.00223	0.0500 c ± 0.00724	0.028 ab±0.00446	0.0270ab±0.00182

Table 3. (continued)

Volatile compound	Corn starch crust	Wheat starch crust	Basmati rice crust	Japonica rice crust	Oat crust	Teff Crust	Quinoa crust	Wheat flour crust
Acetic acid	0.961 a ± 0.114	1.62 bc ± 0.0788	1.55 bc ± 0.130	1.49 b ± 0.126	1.49 b ± 0.0580	2.06 d ± 0.178	2.98 e ± 0.286	1.88 cd ± 0.131
Furfural	0.436 b ± 0.0367	0.437 b ± 0.0255	0.482 bc ± 0.0248	0.541 c ± 0.0185	0.232 a ± 0.00130	2.27 e ± 0.0704	4.58 f ± 0.0714	2.17 d ± 0.0272
2-Ethyl-1-hexanol	0.598 b ± 0.0629	0.602 b ± 0.113	0.536 ab ± 0.0442	0.562 b ± 0.0388	0.515 ab ± 0.00605	0.421 a ± 0.00181	0.425 a ± 0.00735	0.535 ab ± 0.00725
Benzaldehyde	0.0514 a ± 0.00504	0.083 ab ± 0.00903	0.138 bc ± 0.0216	0.141 bc ± 0.00224	0.101 abc ± 0.0123	0.122 abc ± 0.00890	0.166 c ± 0.0710	0.147 bc ± 0.0536
2-(E)-Nonenal	0.0519 bc ± 0.00477	0.023 a ± 0.00167	0.0473 b ± 0.00677	0.0630 c ± 0.00108	0.0463 b ± 0.00374	0.0796 d ± 0.00711	0.0853d ± 0.00922	0.0619 c ± 0.00744
5-Methyl-2-furaldehyde	0.0719 a ± 0.00133	0.088 a ± 0.000283	0.0860 a ± 0.00667	0.0896 a ± 0.00323	0.0760 a ± 0.00461	0.735 c ± 0.0229	1.89 d ± 0.0363	0.414 b ± 0.00920
Butyrolactone	2.59 b ± 0.308	2.08 ab ± 0.409	2.08 ab ± 0.117	1.79 a ± 0.249	2.59 b ± 0.221	4.40 d ± 0.381	4.01 cd ± 0.175	3.54 c ± 0.151
2-Acetilpyrazine	0.0986 a ± 0.00782	0.131 ab ± 0.0320	0.233 cd ± 0.0284	0.140 ab ± 0.0339	0.268 d ± 0.0189	0.198 bc ± 0.0334	0.0953a ±0.000390	0.246 cd ± 0.0487
Butyric acid	0.0507 a ± 0.00442	0.527 b ± 0.0572	0.0515 a ± 0.00144	0.0586 a ± 0.00337	0.0329 a ± 0.00164	0.0550 a ± 0.00586	0.0500±0.000718	0.0460 a ± 0.00252
Phenylacetaldehyde	1.58 e ± 0.174	1.55 e ± 0.0777	0.739 cd ± 0.00331	0.917 d ± 0.0896	0.321 a ± 0.0467	0.662 bc ± 0.0554	0.507 ab ± 0.0534	0.430 a ± 0.0339
Furfuryl alcohol	0.906 c ± 0.0472	1.08 d ± 0.0303	0.845 bc ± 0.00720	0.922 c ± 0.00571	0.538 a ± 0.0101	0.753 b ± 0.0505	2.15 e ± 0.0782	3.24 f ± 0.0649
2-Methylbutyric acid	2.08 a ± 0.323	3.25 b ± 0.677	2.99 ab ± 0.485	3.22 b ± 0.663	2.01 a ± 0.283	3.31 b ± 0.439	2.42 ab ± 0.217	3.38 b ± 0.351

Table 3. (continued)

Volatile compound	Corn starch crust	Wheat starch crust	Basmati rice crust	Japonica rice crust	Oat crust	Teff Crust	Quinoa crust	Wheat flour crust
3-Methylbutyric acid	2.29 ab ± 0.374	2.94 bc ± 0.518	2.90 bc ± 0.545	3.16 c ± 0.421	1.73 a ± 0.153	3.35 c ± 0.229	2.67 bc ± 0.171	3.36 c ± 0.270
2,4-(E,E)-Decadienal	0.0381a± 0.00138	0.147b± 0.0198	0.0395a± 0.00303	0.152b± 0.0195	0.0432a± 0.00147	0.0521a±0.0 0427	0.525c± 0.0568	0.0602 a ± 0.00822
Hexanoic acid	0.0312ab± 0.000241	0.147c± 0.00238	0.0543ab± 0.00689	0.0831b± 0.00375	0.0249a± 0.00144	0.445e± 0.0637	0.271d± 0.0201	0.0480 ab ± 0.000601
Benzyl alcohol	0.853 a ± 0.250	1.20 a ± 0.338	1.44 a ± 0.284	8.01 c ± 0.664	4.66 b ± 0.431	1.070 a ± 0.261	4.91 b ± 0.356	0.827 a ± 0.104
Phenylethyl alcohol	0.772 a ± 0.0742	1.79 c ± 0.108	1.62 c ± 0.0223	1.20 b ± 0.115	0.653 a ± 0.0478	1.10 b ± 0.0265	0.806 a ± 0.0570	2.02 c ± 0.183
2-Acetylpyrrol	2.28 b ± 0.165	1.68 a ± 0.237	2.44 b ± 0.0961	2.57 bc ± 0.260	2.55 bc ± 0.160	3.44 d ± 0.209	4.31 e ± 0.267	2.94 c ± 0.00399
4-Hydroxy-2,5-dimethyl-3(2H)-furanone	0.0484 a ± 0.0127	0.0238 a ± 0.00371	0.0853ab ± 0.00224	0.0719ab ± 0.00324	0.116 bc ± 0.0102	0.160c± 0.000359	0.495 e± 0.0652	0.289 d± 0.0424
4-Vinylguaiacol	0.108 cd± 0.0154	0.0200a ± 0.000557	0.102 bcd± 0.00686	0.0688 abc± 0.00524	0.0487ab ± 0.00266	0.155 d ± 0.00776	0.765 e ± 0.0661	0.0714abc ± 0.00766

Table S1. Peak areas, divided into 10^6, of the 44 studied volatile compounds found with the four fibres tested. The Kovats index (KI) calculated for each volatile compound as well as the KI found in the literature are also given. Different letters in the same row show the significant differences.

Volatile compounds	KI calculated	KI literature	DVB/CAR/PDMS	CAR/PDMS	PDMS/DVB	Polyacrylate
2,3-Butanedione	978	984	1.59 d	1.42 c	0.270 b	nd* a
Hexanal	1060	1080	3.84 c	4.84 d	0.853 b	0.115 a
2-Methyl-1-propanol	1073	1052	0.657 b	1.18 c	0.0659 a	0.0456 a
1-Methylpyrrol	1013	1140	0.0664 c	0.115 c	0.0179 b	nd* a
Heptanal	1141	1168	0.117 b	0.122 b	0.186 c	nd* a
R-Limonene	1154	1202	0.803 c	0.442 b	0.917 d	0.0139 a
Pyrazine	1207	1216	2.80 c	4.61 d	0.685 b	0.103 a
2-Methyl-1-butanol	1207	1218	1.32 b	1.32 b	0.116 a	0.0315 a
3-Methyl-1-butanol	1207	1218	2.94 b	3.31 c	0.288 a	0.0766 a
1-Pentanol	1251	1257	0.449 c	0.697 d	0.141 b	0.0261 a
2-Methylpyrazine	1259	1268	2.66 c	5.83 d	1.83 b	0.175 a
Acetoin	1279	1286	9.29 c	17.9 d	3.74 b	1.96 a
2-Octanone	1279	1283	0.0364 b	0.0945 c	nd* a	nd* a
2,5-Dimethylpyrazine	1315	1316	0.152 b	0.143 b	0.214 c	0.0123 a
2,6-Dimethylpirazine	1321	1319	0.192 b	0.190 b	0.304 c	0.0181 a
2-Ethylpyrazine	1326	1323	0.402 b	0.300 c	0.381 c	0.0261 a
2-Acetyl-1-pyrroline	1326	1325	0.468 c	0.306 b	0.773 d	0.0495 a
2,3-Dimethylpyrazine	1331	1330	0.277 b	0.327 c	0.311 c	nd* a
1-Hexanol	1353	1359	0.182 b	0.416 c	0.198 b	0.0316 a
Nonanal	1388	1396	0.185 c	0.0606 b	0.508 d	0.0315 a
2,3,5-trimethylpyrazine	1395	1396	0.240 c	0.100 b	0.224 c	0.0171 a
2-Ethyl-3-methylpirazine	1395	1400	0.110 c	0.0720 b	0.114 c	0.00862 a
Ethyl octanoate	1432	1437	0.120 b	0.0360 a	0.285 c	0.0277 a
1-Octen-3-ol	1452	1456	2.64 d	1.82 b	2.22 c	0.289 a

Table S1. (continued)

Volatile compounds	KI calculated	KI literature	DVB/CAR/PDMS	CAR/PDMS	PDMS/DVB	Polyacrylate
Acetic acid	1445	1465	15.4 b	22.0 c	3.95 a	4.80 a
Furfural	1461	1467	6.03 c	7.44 d	2.58 b	0.753 a
2-Ethyl-1-hexanol	1490	1489	0.544 c	0.579 c	0.307 b	0.0396 a
Benzaldehyde	1511	1521	0.722 b	0.829 c	1.29 d	0.106 a
2-(E)-Nonenal	1526	1546	1.01 b	0.271 a	2.13 c	0.185 a
5-Methyl-2-furaldehyde	1566	1574	0.251 b	0.304 c	0.348 d	0.0414 a
Butyrolactone	1610	1622	2.09 c	2.45 d	0.697 b	0.432 a
2-Acetilpyrazine	1613	1614	0.120 d	0.0433 b	0.0834 c	0.0263 a
Butyric acid	1623	1636	2.59 c	3.49 d	0.606 b	0.356 a
Phenylacetaldehyde	1630	1642	2.66 b	0.956 a	4.51 c	0.774 a
Furfuryl alcohol	1657	1666	5.75 c	6.92 d	4.21 b	3.15 a
2-Methylbutyric acid	1663	1674	0.943 c	1.19 d	0.351 b	0.150 a
3-Methylbutyric acid	1663	1679	0.768 c	0.900 d	0.210 b	0.104 a
2,4-(E,E)-Decadienal	1797	1797	0.614 c	0.0539 a	1.68 d	0.332 b
Hexanoic acid	1904	1880	4.51 c	3.75 b	4.14 bc	2.10 a
Benzyl alcohol	1962	1893	0.393 c	0.314 b	0.705 d	0.255 a
Phenylethyl alcohol	2041	1942	4.90 c	2.86 a	6.05 d	3.42 b
2-Acetylpyrrol	2168	1950	0.884 b	0.673 a	1.40 c	0.647 a
4-Hydroxy-2,5-dimethyl-3(2H)-furanone	2205	2020	0.500 c	0.0542 a	0.788 d	0.308 b
4-Vinylguaiacol	2253	2230	0.544 b	0.126 a	0.786 c	0.601 b

*nd = not detected

Table S2. Optimisation parameters of the proposed SPME method. The matrix of experimentation as well as the principal components (PC1, PC2) and the desirability of the multiple RSM for each experience are given.

Set	Weight (g)	Time (min)	Temperature (T)	PC1	PC2	Desirability
1	0.500	51.0	50.0	-1.95	1.51	0.542
2	0.750	51.0	50.0	0.21	3.08	0.709
3	0.250	51.0	50.0	-5.44	-1.23	0.259
4	0.500	51.0	70.0	7.36	-4.58	0.000
5	0.500	51.0	40.0	-7.92	-1.08	0.000
6	0.500	75.0	50.0	-0.76	0.42	0.538
7	0.500	30.0	50.0	-4.39	-0.29	0.350
8	0.500	51.0	60.0	4.64	-0.58	0.637
9	0.750	51.0	60.0	8.25	2.73	0.977

Table S3. Correlation parameters found for the volatile compounds that presented odour thresholds (OT) lower than 1 mg Kg^{-1} in the quantitative methodology. The OT lower than 1 mg Kg^{-1} are also given.

Volatile compound	OT (µg Kg^{-1})	PC1* √EV 1 Quantitative	PC2* √EV 2 Quantitative	PC3* √EV 3 Quantitative
2,3-Butanedione (1)	6.50	0.831	0.047	-0.553
Hexanal (2)	4.50	0.511	-0.583	-0.608
1-Methylpyrrol (3)	37.0	0.921	0.101	0.336
Heptanal (4)	3.00	0.809	0.554	0.195
R-Limonene (5)	10.0	-0.850	-0.053	-0.467
Pyrazine (6)	100	0.848	-0.474	0.162
3-Methyl-1-butanol (7)	250	0.293	0.932	0.215
2-Methylpyrazine (8)	105	0.948	0.122	0.075
Acetoin (9)	800	0.316	-0.915	0.251
2-Octanone (10)	50.0	0.550	0.215	0.525
2,5-Dimethylpyrazine (11)	800	0.746	0.626	-0.191
2,6-Dimethylpirazine (12)	200	0.049	0.324	0.801
2-Acetyl-1-pyrroline (13)	0.0530	0.222	-0.311	0.889
Nonanal (14)	1.00	0.922	-0.329	0.127
2,3,5-trimethylpyrazine (15)	400	0.731	0.020	0.088
2-Ethyl-3-methylpirazine (16)	0.400	0.661	-0.630	-0.404
Ethyl octanoate (17)	92.0	0.730	0.390	-0.561
1-Octen-3-ol (18)	1.00	0.928	-0.152	-0.141

Table S3. (continued)

Volatile compound	OT (µg K^{-1})	PC1* √EV 1 Quantitative	PC2* √EV 2 Quantitative	PC3* √EV 3 Quantitative
2-Ethyl-1-hexanol (19)	138	0.106	0.333	0.535
Benzaldehyde (20)	350	0.682	0.321	-0.646
2-(E)-Nonenal (21)	0.0800	-0.894	0.053	-0.441
2-Acetylpyrazine (22)	62.0	0.164	0.378	0.280
Butyric acid (23)	240	0.851	-0.243	-0.105
Phenylacetaldehyde (24)	4.00	0.091	-0.979	0.162
3-Methylbutyric acid (25)	120	0.490	-0.661	0.420
2,4-(E,E)-Decadienal (26)	0.100	0.714	0.228	-0.554
4-Hydroxy-2,5-dimethyl-3(2H)-furanone (27)	30	-0.046	-0.151	0.979
4-Vinylguaiacol (28)	3.00	-0.202	-0.939	-0.252
Acetic acid (29)	30	nq*	nq*	nq*
Furfuryl alcohol (30)	1000	nq*	nq*	nq*

* nq = not quantified

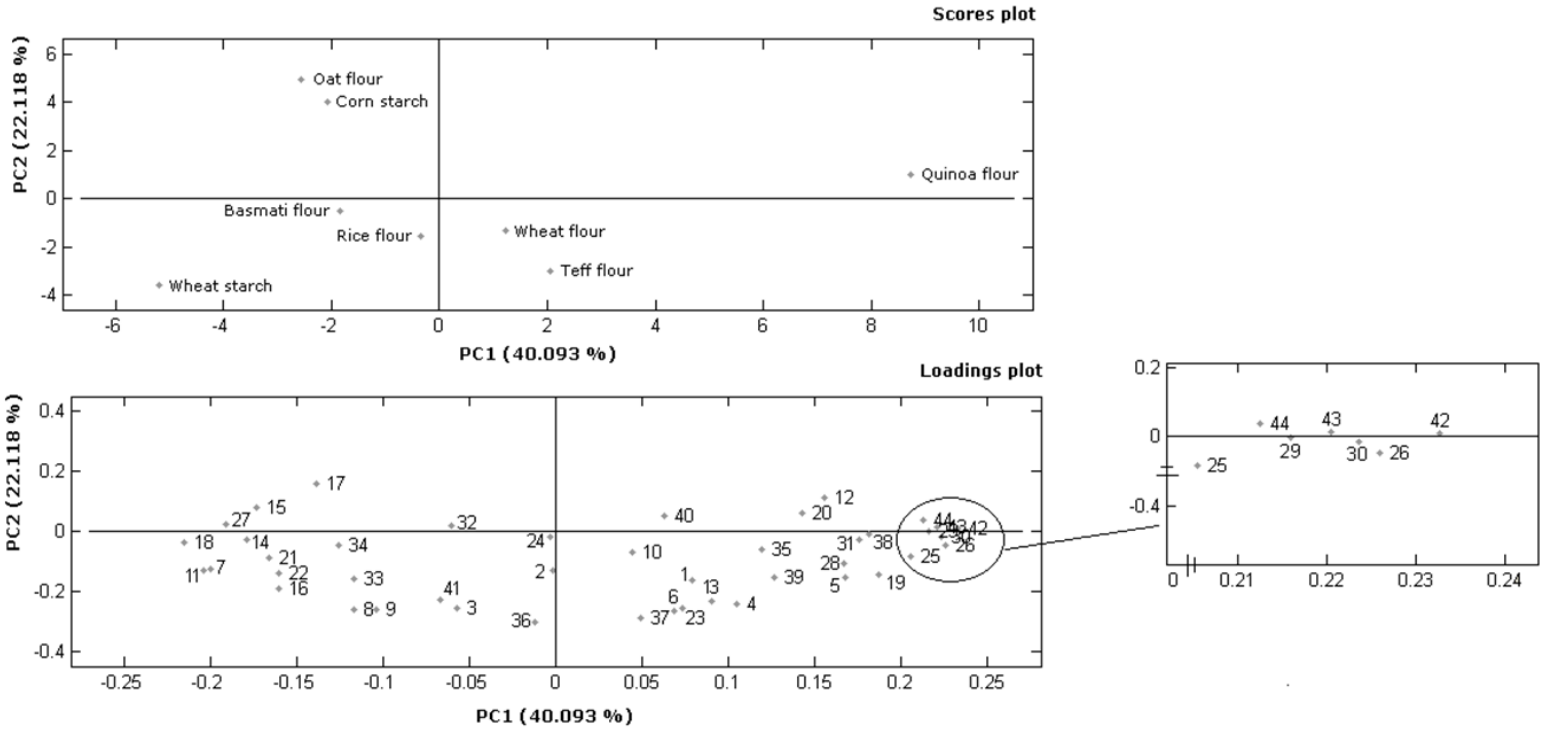

Fig.1. PCA of the gluten-free bread crusts and wheat bread crust (control sample) analysed semi-quantitatively by SPME-GC/QTOF (peak areas represented). The scores plot represents the 8 samples and the loadings plot of the 30 volatile compounds selected for presenting low odour thresholds. The numbers corresponding to each volatile compound are indicated in Table S3.

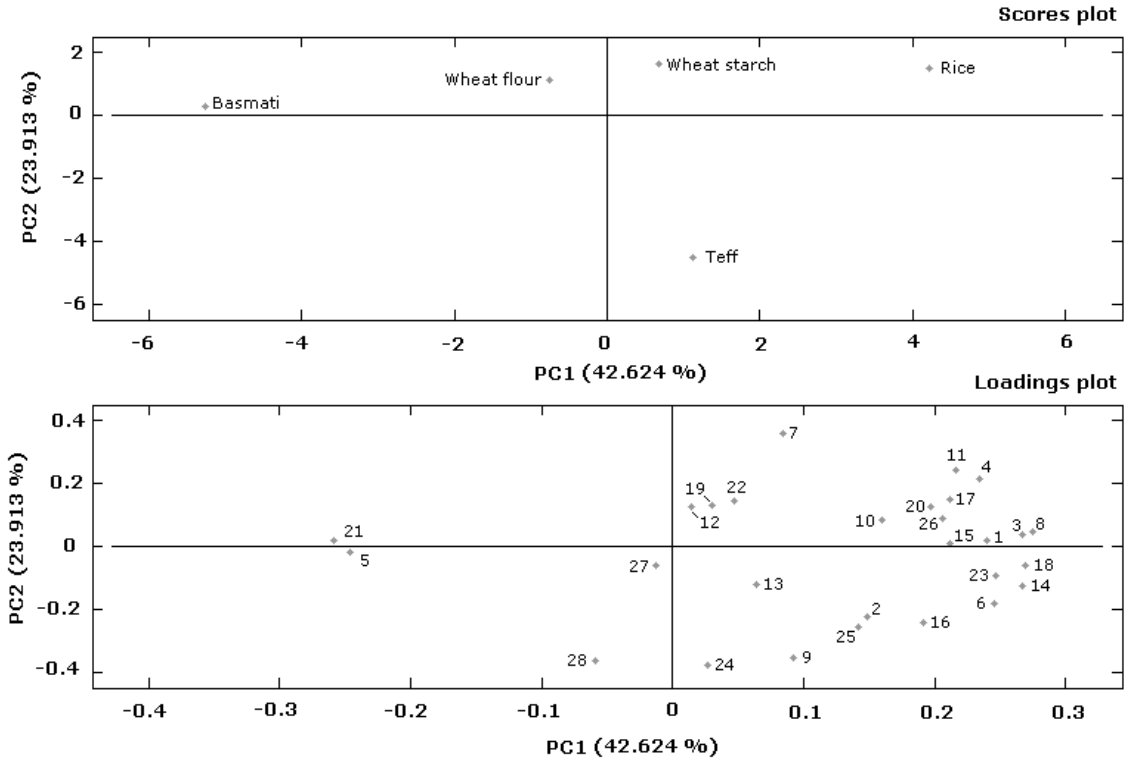

Fig.2. PCA of the gluten-free bread crusts and wheat bread crust (control sample) analysed quantitatively by SPME-GC/QTOF (concentrations represented in µg Kg^{-1}). The scores plot represents the 5 samples and the loadings plot of the 28 volatile compounds quantified. The numbers corresponding to each volatile compound are indicated in Table S3.