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Analytical Methods

An alternative method based on enzymatic fat hydrolysis to quantify volatile compounds in wheat bread crumb

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ABSTRACT

An alternative method to quantify 40 volatile compounds in wheat bread crumb is proposed. It consists of a Soxhlet extraction with a mixture of dichloromethane and diethyl ether containing lipases and a subsequent concentration with Vigreux column. It is the first time that lipases are added to transform the fat into free fatty acids and glycerol, which elute at the end of the chromatogram after the analytes, avoiding problems in the chromatography due to fat residues, such as dirtiness in the injector, column clogging or overlapping peaks. The extract is most easily analysed by GC/MS, using a standard addition method to correct matrix effect. The method was fully validated, with extraction efficiencies between 70% and 100% and precision RSD lower than 15%. The method was applied to a commercial crumb, with acetoin, phenylethyl alcohol and acetic acid as highly abundant compounds, which are considered main volatiles in crumb.

1. Introduction

Bread is one of the most widely consumed foods in the world. It is made with a mixture of flour, water, yeast and salt, all in the right proportions, which is then kneaded, fermented and baked. There are different properties that define its quality, such as volume, texture, colour and flavour, although the aroma of bread is considered essential to its approval by consumers. Analyses of the volatile fraction of bread are very important in order to obtain breads with pleasant smells and also in order to understand the baking processes better.

It is necessary, therefore, to develop analytical methods that allow fast, less expensive and accurate analyses of volatile compounds. There have so far usually been two options to analyse the volatile fraction of breads (Pico, Gómez, Bernal, & Bernal, 2016): solvent extraction methods (Kirchhoff & Schieberle, 2001; Moskowitz, Bin, Elias, & Peterson, 2012; Onishi, Inoue, Araki, Iwabuchi, & Sagara, 2011; Peterson & Jiang, 2013; Rychlik & Grosch, 1996; Zehentbauer & Grosch, 1998) and head space (HS) analyses (Birch, Petersen, Arneborg, & Hansen, 2013; Birch, Petersen, & Hansen, 2013; Maeda et al., 2009; Makoul et al., 2015; Poinot et al., 2007; Ruiz, Quílez, Mestres, & Guasch, 2003). Both methods involve the gas chromatography technique, very often equipped with a mass spectrometry detector (GC/MS). The head space method implies a fast sample treatment with no handling of the matrix, but it contains some disadvantages: HS methods include only the analysis of very volatile compounds (Birch, Petersen, & Hansen, 2014), and in order to obtain high reproducibility, HS techniques require the precise control of many parameters (Birch et al., 2013). Moreover, the amount of isolated volatile fraction is at times so small that important volatile compounds present in bread in low concentrations give no detector signal. Solvent extraction methodologies overcome these drawbacks, but they are time-consuming; the greater the number of steps in sample treatment, the greater the likelihood of analyte losses. Classical solvent extraction techniques have involved extracting the volatile fraction, normally with Soxhlet, and the subsequent concentration with Vigreux columns. However, Soxhlet extraction employs organic solvents that also extract fat from the

Abbreviations: DAGs, diacylglycerides; GC/MS, gas chromatography/mass spectrometry; HS, head space; HVT, high vacuum transfer; LOD, limit of detection; LOQ, limit of quantification; MAGs, monoacylglycerides; MSA, method of standard additions; Qi, qualifier ion i; R^2, determination coefficient; RSD%, relative standard deviation; LipCL, Lipozyme CALB L®; LipPT, Lipase Palatase 20000 L®; LipTL, Lipozyme TL 100 L®; SAFE, Solvent Assisted Flavour Evaporation; SDE, Simultaneous Steam Distillation Extraction; T, target ion; TAGs, triacylglycerides; VS, Vacuum Sublimation.

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bread matrix. Fat could dirty the injector and the GC column and also interact with volatile substances, affecting the recovery percentages (Engel, Bahr, & Schieberle, 1999). Different methods to isolate volatile from non-volatile compounds like fat have emerged: Simultaneous Steam Distillation Extraction (SDE) (Lin, Hsieh, Liu, Lee, & Mau, 2009), Vacuum Sublimation (VS) (Schieberle & Grosch, 1987) and the last method, Solvent Assisted Flavour Evaporation (SAFE) (Engel et al., 1999). Within this context, the use of lipases could represent an alternative to dealing with bread fat. Lipases are enzymes that hydrolyse triacylglycerides (TAGs), diacylglycerides (DAGs) and monoacylglycerides (MAGs) into free fatty acids and glycerol (Murty, Bhat, & Muniswaran, 2002), which are more volatile than TAGs, DAGs and MAGs of fat.

The purpose of the study was to develop and validate an alternative method to analyse volatile compounds in wheat bread crumb by means of lipases, in order to transform the fat by hydrolysis into free fatty acids and glycerol that could elute after the analytes, avoiding chromatographic interferences and instrument dirtiness. As far as we know, this is the first time that lipases have been employed in food aroma analyses with the aim of transforming the fat to avoid the subsequent difficulties in chromatographic determinations.

2. Materials and methods

2.1. Materials, reagents and standards

Dichloromethane and diethyl ether, employed in Soxhlet extraction, were purchased from LAB-SCAN (Gliwice, Poland) and Panreac (Barcelona, Spain), respectively. Methanol used in the standard preparation was obtained from VWR Chemicals (Fontenay-sous-bois, France). Liquid nitrogen used to ground the wheat bread crumb was purchased from Carburos Metálicos (Barcelona, Spain). Enzymes Palatase 20000 L®, Lipozyme TL 100 L® (both from *Aspergillus oryzae*; hydrolyse 1 and 2 bonds) and Lipozyme CALB L® (from *Aspergillus niger*; hydrolyses 1, 2 and 3 bonds) were the lipases tested to hydrolyse fat in the Soxhlet extraction and were all kindly provided from Novozymes (Bagsvaerd, Denmark). The standards employed in the experimental study were all neat and were supplied by Sigma–Aldrich (Steinheim, Germany). They are listed in Table 1 (see Section 2.4).

2.2. Sample description

The method was developed and validated with the crumb of a commercial loaf of wheat bread on the Spanish market. The label showed the ingredients to be wheat flour, water, salt, yeast and flour improver (wheat flour, anti-caking agent (E-170), emulsifier (E-472e), antioxidant (E-300) and enzymes). The loaf of bread weighed 450 g, of which around 130 g were crumb. Sampling is detailed in Section 2.5.

2.3. Preparation of standard solutions

Stock solutions (10,000 mg L^{-1}) of each volatile compound (listed in Table 1) were prepared in methanol. The working solutions were prepared from the mix of stock solutions as required and methanol was used to dilute. All the solutions were stored in a freezer at −21 °C.

2.4. GC–MS instrumentation and chromatographic conditions

GC–MS analyses were performed on a 7890A gas chromatograph (GC) coupled to a 5975C mass spectrometer (MS) detector, which was equipped with a 7683B automatic injector and MS ChemStation 5975C software, all from Hewlett Packard (Palo Alto, California, USA). Separation was achieved on a polar ZB-Wax column (100% polyethylene glycol, 60 m × 0.25 mm ID × 0.25 μm) obtained from Phenomenex (Torrance, California, USA). The GC was operated under programmed temperature conditions ranging from 45 °C (1.5 min) to 100 °C (0 min) at 7 °C/min, after which the temperature was increased to 114 °C (3 min) at 6 °C/min, and then to 136 °C (0 min) at 1.5 °C/min. Finally, the temperature was raised to 245 °C at 85 °C/min. This was held for 8 min in the case of standard solution injection, but for 25 min for wheat bread crumb samples (as explained in Section 3.1.1). Total run time was 48 min and 65 min, respectively. An injection volume of 1 μL was employed with the autosampler in pulsed splitless mode. The inlet temperature was set at 250 °C and the carrier gas was Helium supplied by Carburos Metálicos (Barcelona, Spain) at a flow rate of 1.1 mL/min. The interface, ion source and quadrupole temperatures were 250 °C, 230 °C and 150 °C, respectively. The MS scan parameters included a mass range of 15–350 m/z, operating in positive electron impact mode with ionisation energy of 70 eV. Analyses were performed with selected ion monitoring mode (SIM), with one target (T) and two qualifier ions (Q_1 and Q_2) for each volatile compound (see Table 1). The 40 analytes were identified and confirmed by a comparison of their retention times and mass spectra with standards and the Mass Spectra Library (Wiley 7N edition).

Table 1
Volatile compounds studied in the validated method in order of elution. Target (T) and qualifier (Q_1, Q_2, +Q) ions employed for each compound are given in the table. The numbering used for the peaks in Fig. 2 is also given in the last column.

Volatiles	T	Q_1	Q_2	+Q	Peak label
2,3-Butanedione	43	86	15	42	1
1-Propanol	31	42	59	60	2
2-Methyl-1-propanol	43	41	74	55	3
Hexanal	44	56	72	82	4
3-Penten-2-ol	71	43	53	86	5
2-Methyl-1-butanol	57	41	70	29	6
3-Methyl-1-butanol	55	70	41	57	7
R-(+)-limonene	68	93	79	107	8
Ethyl hexanoate	88	99	43	60	9
1-Pentanol	42	55	70	91	10
Acetoin	45	88	27	15	11
2-Octanone	58	71	85	128	12
1-Hydroxy-2-propanone	43	31	74	29	13
Ethyl lactate	45	75	29	19	14
1-Hexanol	56	43	69	84	15
Nonanal	57	41	70	98	16
Acetic acid	45	60	15	29	20
1-Octen-3-ol	57	72	43	85	18
Methional	48	104	76	61	19
Furfural	96	39	29	67	21
Ethyl octanoate	88	101	127	57	17
2-Ethyl-1-hexanol	57	41	70	83	22
Benzaldehyde	106	105	77	51	23
2,3-Butanediol	45	57	29	75	24
2-(E)-nonenal	70	55	41	83	25
Isobutyric acid	43	41	73	27	26
5-Methyl-2-furaldehyde	110	109	53	81	27
1,2-Propanediol	45	43	61	29	28
Butyric acid	60	73	42	27	29
Butyrolactone	42	28	86	56	30
Phenylacetaldehyde	91	120	92	65	31
Furfuryl alcohol	98	81	53	69	32
2-Methylbutanoic acid	57	74	87	41	33
3-Methylbutanoic acid	60	43	87	39	34
1,3-Butanediol	43	45	57	72	35
2,4-(E,E)-decadienal	81	67	95	152	36
Hexanoic acid	60	73	87	41	37
Benzyl alcohol	79	108	91	51	38
Phenylethyl alcohol	91	122	65	77	39
4-Vinylguaiacol	150	135	107	77	40