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Evolution of volatile compounds in gluten-free bread: From dough to crumb

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ABSTRACT

Understanding the evolution of volatile compounds from dough to crumb is necessary in order to improve the weak aroma of gluten-free breads. Additionally, sensitive analytical methods are required to detect small changes. In the present study, a solvent extraction method combined with GC/MS was selected to examine the evolution of 31 principal volatile compounds from the beginning of fermentation to the end of baking in maize starch bread. During fermentation, only hexanal, hexanoic acid, benzaldehyde, benzyl alcohol, furfural and furfuryl alcohol remained constant whereas the rest became more abundant. After baking, 2,3-butanedione, 1-propanol, 2-methyl-1-propanol, 3/2-methyl-1-butanol and ethyl octanoate were evaporated whereas the other volatile compounds increased. The alcohols from fermentation, 2,3-butanedione, acetoin, acetic acid, isobutyric acid and ethyl octanoate, were the main volatile compounds in dough; all of them were formed during fermentation. In crumb, alongside those compounds, hexanal, 1-octen-3-ol and nonanal, produced from lipid oxidation, were also important contributors.

1. Introduction

The aroma of bread is one of the main characteristics that influences the customers' choice. There can be no doubt that the ingredients of the recipe should affect the final aroma of bread. In fact, when the bread is elaborated with gluten-free flours, the resulting aroma is weaker than those elaborated with wheat or rye (Pacyński, Wojtasiak, & Mildner-Szkudlarz, 2015). The processes that lead to the final aroma of bread, such as fermentation, lipid oxidation or Maillard reactions, strongly depend not only on the recipe but also on the fermentation and baking conditions (Cho & Peterson, 2010). Thus, it is really important to determine the evolution of the volatile compounds from the 0 min fermented dough to the fermented dough and finally to the baked bread, in order to understand their generation in the different steps. Therefore, the processing of gluten-free breads could be modified to achieve a stronger, improved aroma. However there are only a few studies regarding the aroma of gluten-free breads. Furthermore, they analyse the crumb and crust together without specifying where the volatile compounds come from (Aguilar, Albanell, Miñarro, Gallardo, & Capellas, 2015; Poinot et al., 2009). To our knowledge, there is no research regarding the aroma of gluten-free doughs and its evolution to the crumb. Only one research study regarding the evolution of volatile compounds was found, but in wheat bread (Makhoul et al., 2015).

The selected analytical technique is also important because trace analyses are necessary to detect small differences between the different steps of bread production and, above all, between the different stages of fermentation. Solvent extraction methodologies possess in general lower limits of detection than methodologies using headspace (Pico, Gómez, Bernal, & Bernal, 2016) and allow the detection of a higher number of compounds (Majcher & Jeleń, 2009). The most employed extractant is dichloromethane (Gassenmeier & Schieberle, 1995; Schieberle & Grosch, 1994; Zehentbauer & Grosch, 1998) and only for a few compounds, normally acidic compounds, diethyl ether is also reported (Zehentbauer & Grosch, 1998). Moreover, solvent extraction methodologies have been shown to result in extracts rich in high-molecular weight volatile compounds whereas solid-phase microextraction (SPME) to result in extracts rich in low-molecular weight volatile compounds (Mayuoni-kirshinbaum,

Abbreviations: ANOVA, analysis of variance; FD, flavour dilution factor; GC/MS, gas chromatography/mass spectrometry; HPMC, hydroxypropyl methylcellulose; OAV, odour activity value; OT, odour threshold; PCA, principal component analysis; PC1, first principal component; SIM, selected ion monitoring.

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Tietel, Porat, & Ulrich, 2012). Thus, SPME may be beneficial for volatile compounds that are very volatile and co-elute with the solvent. On the other hand, solvent extraction treatments are more tedious and need the use of organic solvents (Pico et al., 2016). Therefore, solvent extraction methodologies and headspace methodologies have been frequently employed in a complementary way (Corral, Salvador, & Flores, 2015; Klensporf & Jeleń, 2008; Majcher & Jeleń, 2009). With the purpose of detecting small changes in the concentration of the most important compounds, a solvent extraction method for volatile compounds in bread with low limits of detection was selected.

Therefore, the goal of this study was to understand and explain the evolution of the most common volatile compounds in gluten-free breads from fermented dough after 0 min, 45 min and 90 min to the baked crumb employing a sensitive solvent extraction – GC/MS methodology. In this way it would be possible to tentatively establish conclusions about ways to improve the aroma of gluten-free breads.

2. Materials and methods

2.1. Materials

Maize starch (Miwon Daesang, Seul, Korea), hydroxypropyl methylcellulose (HPMC) (Methocel™ K4M, Dow Chemicals, Midland, MI) and *Saccharomyces cerevisiae* (Saf-instant yeast) (Lesaffre, Lille, France) were used. Sucrose, salt and sunflower oil were purchased from the local market and tap water was employed.

To check the retention time and the mass spectra of the main volatile compounds, the 31 analytical standards listed in Table 1 were purchased from Sigma-Aldrich (Gillingham, UK). The purity of all the standards was higher than 98%.

2.2. Methods

2.2.1. Gluten-free bread making

The following ingredients, as a % on starch basis, were employed: sunflower oil (3%), sucrose (5%), salt (1.8%), instant yeast (3%), HPMC (2%) and water (100%). The dough was elaborated with a basis of 700 g (±0.05 g) of starch and the amount of starch and water was adjusted to an average moisture content of 12%. The ingredients were mixed using a Kitchen-Aid Professional mixer (KPM5; KitchenAid, St. Joseph, MI) for 8 min at speed 56 rpm.

Four aluminum tins were filled with 100 g (±0.05 g) of kneaded dough. 5 mL of a mixture of methyl octanoate and methyl decanoate (20 g L^{-1}, dimethyl sulfide) were added to one of them (0 min fermented dough) in order to inhibit the fermentation evolution (data not shown) and finally it was frozen at −20 °C. The other two were left to ferment for 45 min and 90 min in a chamber at 30 °C with 90% of humidity and then their fermentation inhibition was performed as explained (45 min fermented dough and 90 min fermented dough). The frozen doughs were left at room temperature 30 min before their aroma analyses. The last sample was left to ferment for 90 min and then baked at 190 °C for 40 min. After baking, the gluten-free bread was left at room temperature for 30 min and cut into loaves of 5 cm long. The crumb was separated from 1 cm to crust, to avoid the crumb contamination with crust volatile compounds. Finally, the crumb was ground and frozen at −20 °C. The frozen crumb was left at room temperature 30 min before its aroma analysis. Each dough and crumb was prepared in duplicate (n = 2).

2.2.2. Volatile compounds analyses: solvent extraction & GC/MS

The 0 min, 45 min and 90 min fermented doughs as well as the crumb were analysed following the lipases extraction method (Pico, Nozal, Gómez, & Bernal, 2016): 50 g of each sample were ground with liquid nitrogen and then submitted to a Soxhlet extraction for 5 h at 40 °C with a mixture of diethyl ether/dichloromethane, which contained 25 μL lipase enzyme (Lipozyme CALB L©) in order to hydrolyse the fat. After that, the extract was concentrated by means of a Vigreux column and injected onto the GC/MS. It is a suitable method to examine the evolution of volatile compounds from dough to crumb, since the reported limits of detection were lower than 35 μg kg^{-1}. Analyses were performed in duplicate ($n = 2$).

GC–MS analyses were performed on a 7890A gas chromatograph coupled to a 5975C single quadrupole mass spectrometer detector equipped with a 7683B automatic injector and Chemstation 5975C software, all from Agilent Technologies (Santa Clara, CA). 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, CA). The gas chromatograph 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. It was held for 25 min in order to elute the hydrolyzed fat (glycerol and free fatty acids). The carrier gas was also helium, 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. Analyses were performed in SIM mode, operating in positive electron impact mode with ionization energy of 70 eV. All the volatile compounds were identified and confirmed by comparison of their retention times and mass spectra (target and qualifier ions) with standards (Table 1) and with the Wiley 7 N edition mass spectral library. Firstly, the standard corresponding to each volatile compound was injected individually in order to unequivocally determine its retention time. After that, the mixture of the 31 standards was injected to check the final retention time. This mixture of standards was injected simultaneously with each sample. The retention time of most of them was slightly different from dough to crumb (1–1.5 min of delay in dough) due to the different matrix and the presence of inhibitor substances in the dough.

2.2.3. Data analysis

In order to better represent and interpret the results of the evolution of each volatile compound in the different samples (0 min, 45 min and 90 min fermented doughs and the crumb), a one-way analysis of variance of the peak areas was calculated using Statgraphics Centurion version XVII (Statpoint Technologies, Warrenton, VA). The total number of replicates was four ($n = 4$), with the dough and crumb prepared in duplicate and, in turn, analysed in duplicate. Principal component analysis (PCA) of the three doughs was calculated with the software Latentix (version 2.00, Latent5), with all GC/MS data autoscaled prior to the analysis.

3. Results and discussion

3.1. Evolution of the main volatile compounds during fermentation

A total of 31 volatile compounds was found and examined to understand their generation and evolution from dough to crumb in maize starch bread, integrating the peak area of each volatile compound. These 31 volatile compounds have been commonly reported in wheat bread dough (Martínez-Anaya, 1996) and crumb (Birch, Petersen, & Hansen, 2014; Pico, Bernal, & Gómez, 2015). Volatile compounds, like 3/2-methyl-1-butanol, benzyl alcohol, phenylethyl alcohol, phenylacetaldehyde, 2,3-butanedione, acetoin and 3-methylbutanoic acid, have been reported to have a positive impact in the final aroma of wheat bread, whereas 1-octen-3-ol,