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ORIGINAL ARTICLE

Influence of different flours and starches on gluten-free bread aroma

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Abstract The aim of this research was to study the effect of different gluten-free flours (yellow and white corn, rice, oat, teff, buckwheat, amaranth and quinoa) and starches (wheat, corn and potato) on the generation of volatile compounds in the fermented doughs and crumbs. Volatile compounds were analyzed by static headspace-gas chromatography/mass spectrometry (SHS-GC/MS). Nine fermentation and lipid oxidation volatile compounds were evaluated, which were found to be the same from dough to crumb but vary in levels. Concentrations of compounds produced during fermentation were higher in doughs whereas those from lipid oxidation were higher in crumbs. The type of flour/starch affected the concentration of these volatile compounds. The proportions of ethanol and 2/3-methylbutanol (fermentation compounds) were higher in dough from yellow and white corn, rice and oat while the proportions of hexanal, 1-pentanol and 2,4-decadienal (lipid oxidation compounds) were higher in the doughs made with starches. The proportions of ethanol and 2/3-methylbutanol were higher in quinoa and amaranth crumbs whilst hexanal, 1-pentanol and 2,4-decadienal were higher in yellow and white corn crumbs.

Keywords Volatile compounds · Gluten-free flours · Crumb aroma · Dough aroma · Fermentation · Lipid oxidation

Abbreviations

HPMC	Hydroxymethyl-propyl-cellulose
PCA	Principal component analysis
PC1	First principal component
PC2	Second principal component
SD	Standard deviation
SHS-GC/MS	Static headspace extraction-gas chromatography/mass spectrometry
SIM	Selected ion monitoring
T	Target ion

Introduction

Bread aroma is one of the first characteristics perceived by the human senses, crucial for the acceptance by customers. The most consumed breads have been prepared with wheat or rye flours, which give pleasant notes with compounds that come mainly from fermentation, lipid oxidation or Maillard processes. However, it is well known that if the bread is elaborated with gluten-free flours, its sensory quality decreases in relation to the traditional wheat bread. Celiac people can only consume gluten-free products, which means that they should eat breads with less attractive flavors. Cereal flours such as rice, corn, millet, and teff and gluten-free starches have been commonly employed during gluten-free bread making (Pacyński et al. 2015). Gluten-free breads have been also elaborated with pseudocereals like buckwheat, quinoa or amaranth. Pseudocereals have been reported to contain high nutritional values in terms of proteins, lipids, carbohydrates, vitamins, minerals and fiber

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(Alvarez-Jubete et al. 2010; Hirose et al. 2010; Jancurová et al. 2009). Moreover, they present higher α-glucosidase activity (Elgeti et al. 2014) but lower lipoxygenase activity (Caussette et al. 1997) than conventional wheat flour. Their use has probably not been extended due to their content in saponins, which give bitter taste notes to bread (Oleszek et al. 1999), and also due to their lower availability and higher price.

The differences in proteins, sugars, lipids, enzymes and antioxidants between the different gluten-free flours/starches could lead to important differences in the volatile profile of gluten free breads. Until now, research in gluten-free bread aroma has been focused on the understanding of the origin of the volatile compounds compared to wheat bread (Poinot et al. 2009). There are also a few articles regarding the improvement of gluten-free bread aroma based on the method of baking (Aguilar et al. 2015) or on the addition of sugar-amino acid pairs to encourage Maillard reaction (Pacyński et al. 2015). Nevertheless, as far as we know, there have not been studies of the influence on gluten-free bread aroma of different flours or starches. All the reported articles refer to a mixture of gluten-free flours [rice, corn and buckwheat flours with corn and potato starches (Poinot et al. 2009) or corn starch with chickpea flour (Aguilar et al. 2015)] or to a commercial preparation based on starches (Pacyński et al. 2015).

The aim of this research was to study the effect of different gluten-free flours (yellow and white corn, rice, oat, teff, buckwheat, amaranth and quinoa) and starches (corn, wheat and potato) affect the generation of volatile compounds in the corresponding bread dough and crumb. The volatile compounds generated through the fermentation and lipid oxidation processes were analyzed by static headspace extraction-gas chromatography/mass spectrometry (SHS-GC/MS), since they are considered the main aroma compounds in bread dough and crumb. SHS enables the direct measurement of the ratio of the most abundant compounds in the gaseous phase (Maeda et al. 2009). Both fermented dough and crumb were analyzed in order to understand the influence of bread processing through the subsequent changes of the volatile profile from dough to the related crumb. This could lead to a better understanding of the impact of changing the flour/starch on the aroma of gluten-free breads.

As our knowledge, it is the first time that the aroma profiles of different gluten-free doughs and crumbs elaborated only with one flour or starch have been compared. Knowing the influence of the flour or starch could be essential to producing gluten-free breads with an improved aroma.

Materials and methods

Recipe ingredients: flours, starches, hydrocolloid and yeast

Wheat and potato starches were supplied by Roquette Laisa (Valencia, Spain) and corn starch by Miwon Daesang (Seul, Korea). Wheat flour was purchased from Harinera Castellana (Medina del Campo, España), yellow and white corn flour from Dacsa (Valencia, Spain), rice flour from Molendum (Zamora, Spain), oat flour from Emilio Esteban (Valladolid, Spain) and teff flour from Salutef (Palencia, Spain). Buckwheat, amaranth and quinoa flours were obtained from El Granero Integral (Madrid, Spain). Hydroxyl propyl methyl cellulose (HPMC) 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.

Bread making

The following ingredients (as % on flour or starch basis) were used in all the formulas: sunflower oil (6%), sucrose (5%), salt (1.8%), instant yeast (3%), HPMC (2%) and water (100%). The doughs were elaborated with a basis of 700 g (±0.05 g) of each flour or starch and the contents of flour/starch and water were adjusted to an average moisture content of 12%. They were mixed using a Kitchen-Aid Professional mixer (KPM5, KitchenAid, St. Joseph, Michigan, USA) for 8 min at speed 2. From each dough, 100 g (±0.05 g) were transferred to aluminum tins and left for fermentation for 90 min in a chamber at 30 °C with 90% of humidity. Half of the fermented dough was separated and prepared for volatile compounds analysis, which means the stop of the residual fermentation and final freezing prior to SHS analysis (Martínez-Anaya et al. 1990). The other half of the dough was baked at 190 °C for 40 min. After baking, the gluten-free breads were 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 (Birch et al. 2013). Finally, the crumbs were grounded and frozen at −20 °C in packages of 4 g prior to SHS-GC/MS analysis. Wheat bread dough and crumb samples were employed as control samples, since it is the most commonly consumed bread. In the wheat bread recipe there was no addition of HPMC, the rest of the ingredients and bread making conditions were identical. In order to understand the losses of fermentation volatile compounds during baking regarding the structure, the bread volumes

were determined. Bread volumes were measured 24 h after baking, by duplicate (n = 2) using a laser sensor with the Volscan Profiler volume analyser (Stable Micro Systems, Surrey, UK).

Standards and solvents

To check the retention times and the m/z of the target ions, the following standards were purchased from Sigma Aldrich (Gillingham, UK): hexanal, 2-methylbutanol, 3-methylbutanol, 1-pentanol, 2-heptenal, hexanoic acid, acetaldehyde, 3-methylbutanal, 2,4-decadienal. Acetone and ethyl alcohol were supplied by Panreac (Barcelona, Spain).

Sample procedure: static headspace extraction (SHS)

The frozen samples (crumb and 90 min fermented dough) were tempered at room temperature during 30 min. Thereafter, 1 g (±0.050 g) of each sample (dough or crumb) was introduced in a 20 mL vial and sealed with a septum cap. After that, the sample was extracted for 90 min at 90 °C, without agitation, in a Static Headspace autosampler 7694 from Hewlett Packard (Palo Alto, California, USA). The loop and transfer line temperature were, respectively, 100 and 105 °C. The carrier gas employed was helium, supplied by Carburos Metálicos (Barcelona, Spain), with a carrier gas pressure of 23 psi and the vial pressurization was 14 psi for 0.2 min. The loop filling time was 0.2 min, the equilibration loop time was 0.05 min and the injection time 1 min. Each sample was analyzed in triplicate (n = 3).

Gas chromatography–mass spectrometry (GC–MS) conditions

GC–MS analyses were performed on a 7890A gas chromatograph coupled to a 5975C mass spectrometer detector (single quadrupole) equipped with a 7683B automatic injector and a 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 (New South Wales, Australia). The GC was operated under programmed temperature conditions from 45 °C (1.5 min) to 100 °C (0 min) at 7 °C/min, afterwards temperature was increased to 114 °C (6.7 min) at 1 °C/min. The total run time was 30 min. The carrier gas was also 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, 230 and 150 °C, respectively. The MS scan parameters included a mass range of 15–350 m/z, operating in positive electron impact mode with ionization energy of 70 eV. Analyses were performed with selected ion monitoring mode (SIM), with one target (T) and two quantifier ions (Q_1 and Q_2) for each of the volatile compounds. The sixteen analytes were identified and confirmed by comparison of their retention times and mass spectra with standards and with the Mass Spectra Library (Wiley 7 N edition).

Data analysis

Principal component analysis (PCA) was calculated with the software Latentix (version 2.00, Latent5), with all GC/MS data autoscaled prior to the analysis. PCA is a suitable technique to describe major trends in a group of data and to detect possible outliers (Birch et al. 2013). The new variables (principal components) are constructed from a data matrix of the samples, where the scores are related to the samples (bread doughs and crumbs) and the loadings are related to the variables (volatile compounds). A large portion of the variability is often described by a few principal components. In this article, the scores plot and loadings plot are employed, showing the relationship between high/low values of the variables and the samples.

Results and discussion

Identification of volatile compounds in gluten-free bread doughs and crumbs

Sixteen main volatile compounds were identified both in dough and crumb in twelve breads by comparing with standards of their retention times and their target and two qualifier ions. As it is shown in Table 1, there were volatile compounds produced during fermentation and lipid oxidation and some of these by both (fermentation and lipid oxidation or fermentation and Maillard) (Pico et al. 2015). The study of the Maillard compounds in doughs and crumbs was not taken into consideration, since it was not possible to know if these were increased with the SHS temperatures. However, Maillard compounds are considered crucial only for the crust and the purpose of this study was to examine the influence of different flours and starches on the generation of volatile compounds in gluten-free doughs and crumbs.

Therefore, from the sixteen volatile compounds identified both in dough and crumb, acetone, ethyl alcohol, hexanal, 2-methyl-1-butanol, 3-methyl-1-butanol, 1-pentanol, 2-heptenal, hexanoic acid and 2,4-decadienal were evaluated (in bold in Table 1).

Table 1 Main volatile compounds identified in all gluten-free bread doughs and crumbs

Volatile compounds	Source
Acetone	**Fermentation**
Ethyl alcohol	**Fermentation**
2-Methyl-1-butanol	**Fermentation**
3-Methyl-1-butanol	**Fermentation**
Hexanal	**Lipid oxidation**
1-Pentanol	**Lipid oxidation**
2-Heptenal	**Lipid oxidation**
2.4-Decadienal	**Lipid oxidation**
Hexanoic acid	**Fermentation & Lipid oxidation**
Acetaldehyde	Fermentation and Strecker degradation
3-Methylbutanal	Fermentation and Strecker degradation
2.3-Butanedione	Fermentation and Maillard
Acetoin	Fermentation and Maillard
Acetic acid	Fermentation and Maillard
Furfural	Fermentation and Maillard
Furfuryl alcohol	Fermentation and Maillard

Effect of the flour/starch on the different gluten-free bread doughs' volatile compounds

The results of nine fermentation and lipid oxidation compounds found in the doughs are given in area signal of the target ion (Table 2). Regarding these compounds, all twelve gluten free doughs present the same fermentation and lipid oxidation compounds, though in different concentration. Taking into consideration that only the kind of flour/starch was changed between the different doughs, keeping the rest of the recipe identical, this could led to conclude that the type of flour/starch affected the concentration of the fermentation and lipid oxidation compounds but not the creation/elimination of these (regarding the compounds detected with SHS-GC/MS, since only the most abundant compounds are present).

For an overview of the influence of the flour/starch on the generation of volatile compounds in the doughs, PCA was done for the data obtained (peak areas) (Fig. S1). Thus, it was possible to understand if the use of certain flour involved an increase or a decrease in the concentration of the volatile compounds that came from fermentation or lipid oxidation processes in relation to the other flours. The first principal component (PC1) explained 40.5% of the variability of the original variables. Regarding the scores plot, there was a clear separation between starches (negative x-axis) and flours (positive x-axis) and with regard to the loadings plot, the variables were clearly divided into lipid oxidation compounds (negative component of PC1) and fermentation compounds (positive

Table 2 Fermentation and lipid oxidation volatile compounds identified in all doughs

	Acetone	Ethyl alcohol	Hexanal	2-Methyl-1-butanol	3-Methyl-1-butanol	1-Pentanol	2-Heptenal	Hexanoic acid	2.4-Decadienal
Wheat starch	3.56 ± 0.31	865.23 ± 105.12	27.12 ± 2.27	2.17 ± 0.20	4.14 ± 0.35	6.12 ± 0.72	2.39 ± 0.26	4.21 ± 0.28	2.31 ± 0.21
Corn starch	3.50 ± 0.25	488.82 ± 60.82	17.08 ± 1.07	2.03 ± 0.25	3.19 ± 0.21	2.75 ± 0.39	2.87 ± 0.29	0.00 ± 0.00	1.88 ± 0.16
Potato starch	3.46 ± 0.28	786.14 ± 87.39	4.67 ± 0.54	2.58 ± 0.31	4.10 ± 0.39	0.61 ± 0.04	0.41 ± 0.04	4.70 ± 0.36	3.74 ± 0.33
Wheat flour	4.37 ± 0.38	519.36 ± 74.25	9.23 ± 0.95	0.95 ± 0.13	1.76 ± 0.18	2.67 ± 0.39	0.57 ± 0.07	2.58 ± 0.19	1.90 ± 0.16
Yellow corn flour	12.71 ± 1.07	1070.17 ± 130.32	11.31 ± 1.15	3.84 ± 0.46	6.44 ± 0.52	2.29 ± 0.30	0.82 ± 0.09	4.53 ± 0.30	1.70 ± 0.12
White corn flour	18.83 ± 1.66	853.03 ± 101.72	18.28 ± 1.48	2.90 ± 0.31	5.27 ± 0.48	4.94 ± 0.61	0.70 ± 0.04	1.51 ± 0.12	0.78 ± 0.09
Buckwheat flour	14.41 ± 1.14	707.91 ± 85.03	1.02 ± 0.07	3.35 ± 0.35	5.48 ± 0.45	0.27 ± 0.04	0.15 ± 0.01	1.21 ± 0.07	0.98 ± 0.06
Rice flour	9.77 ± 0.89	905.88 ± 110.12	5.77 ± 0.59	3.42 ± 0.50	5.54 ± 0.41	0.98 ± 0.16	0.47 ± 0.06	4.87 ± 0.35	1.15 ± 0.15
Oat flour	8.11 ± 0.60	1024.37 ± 124.83	5.00 ± 0.37	3.77 ± 0.42	6.13 ± 0.56	1.98 ± 0.21	0.40 ± 0.02	3.72 ± 0.22	1.86 ± 0.14
Teff flour	19.16 ± 0.35	763.07 ± 87.55	1.34 ± 0.16	2.66 ± 0.28	4.38 ± 0.28	0.14 ± 0.09	0.29 ± 0.01	2.34 ± 0.26	1.64 ± 0.25
Quinoa flour	18.80 ± 1.49	607.07 ± 92.46	2.74 ± 0.15	3.24 ± 0.32	7.91 ± 0.32	0.37 ± 0.02	0.13 ± 0.05	1.29 ± 0.18	2.36 ± 0.13
Amaranth flour	13.08 ± 1.56	525.97 ± 75.13	1.69 ± 0.25	2.54 ± 0.37	3.87 ± 0.69	0.42 ± 0.06	0.20 ± 0.01	1.40 ± 0.07	0.75 ± 0.27

Results are given in area values ($\times 10^5$). Standard deviations (SD) are given after ± (n = 3)

component of PC1). Therefore, the separation of the gluten-free doughs between flours and starches could be attributed to the higher content in fermentation compounds of the flours and in lipid oxidation compounds in the case of starches. With respect to the Table 2, the measured contents of hexanal, 1-pentanol and 2-heptenal were especially higher in wheat and corn starch doughs than in flour doughs (except white corn flour). This could be surprising due to the contents of lipids in wheat and corn starch, which were reported to be lower than in flours, being in quinoa and amaranth flours the highest (as can be seen in Table S1, see supplementary data). However, not only the content of lipids should be taken into consideration. Lipoxygenase activity in flours also determines the amount of lipids that are susceptible to oxidation. With regard to starches, the most important involved enzymes have been amylases, glucoamylases and phosphorilases (BeMiller and Whistler 2009), but lipoxygenases have not been reported. It is supposed that the amount of lipoxygenases in starches caused by contamination of its isolation from flour could be negligible. Nevertheless, the main source of hydroperoxides decomposition that led to lipid oxidation compounds in storage food, like flour, were non-enzymatic reactions instead of enzymes ones (Gardner 1975). It implied a homolytic cleavage of the hydroperoxy group in a free-radical mechanism promoted by heat, photolysis, metal ions and other agents that promote free-radicals (Gardner 1975). Therefore, although lipoxygenase activity is not expected in starches, non-enzymatic lipid oxidation processes may have led to a higher concentration of lipid oxidation volatile compounds, such as hexanal, related to flours. In addition, cereals, pseudocereals and the corresponding flours contained a significant amount of antioxidants, like vitamin E and flavonoids, which trapped the hydroperoxides diminishing the final oxidation rate of the lipid oxidation. The amount of vitamin E (Alvarez-Jubete et al. 2010) and flavonoids (Hirose et al. 2010) has been reported to be much higher in pseudocereals, such as quinoa, than in cereals (Laus et al. 2012). However, antioxidant substances have not been reported in starches. Therefore, as the storage time of the flours/starches is also crucial (Maraschin et al. 2008), a balance between the lipid oxidation processes and the action of antioxidants should be considered to understand their content in volatile compounds from lipid oxidation processes.

Regarding the second principal component (PC2), it explains 26.7% of the variability. In the case of starches, wheat starch appears in the positive component of PC2 meanwhile corn starch in the negative axis of PC2. This means that in wheat starch there is a higher amount of volatile compounds from lipid oxidation than in corn starch. This is in concordance with those reported by Blaszczak et al. (2003), who found that wheat starch contains the highest content of total lipids, followed by corn starch and potato starch.

Taking into account the PC2 for gluten free flours, there is a clear separation between common gluten-free cereal flours (positive component of PC2) and pseudocereal flours (negative component of PC2). However, although teff belongs to the cereal crops family such as corn, rice and oat, it is in the same area of pseudocereals. It may be related to the chemical composition of teff flour, since the whole grain was milled as in pseudocereal flours, while the other flours were white flours. Their separation in the PC2 may be associated to the higher content of ethanol (fermentation marker) in corn, rice and oat doughs. As saccharose was added in the gluten-free breads, free sugars may not be the only limiting factor. Elgeti et al. (2014) reported that the α-glucosidase activity in quinoa flour was higher than in corn, rice or wheat flour. The release of fermentable sugars during fermentation could be partially attributed to the action of α-glucosidase and therefore the content of ethanol was higher in quinoa or amaranth dough. However, it has also been reported that flavonoids could act as inhibitors of α-glucosidase activity (Giménez-Bastida and Zieliński 2015; Li et al. 2009). The content of antioxidants, such as flavonoids, has been reported much higher in pseudocereals (Hirose et al. 2010) than in cereals like wheat (Giménez-Bastida and Zieliński 2015). This could explain the lower content in ethanol measured in these pseudocereals dough related to corn, rice and oat dough (Table 2). However, in the case of 3-methyl-1-butanol and 2-methyl-1-butanol, important markers of fermentation, the differences between cereal flours and pseudocereal flours were not so large, being the lowest in wheat flour. These compounds are Ehrlich alcohols generated during fermentation from leucine and isoleucine amino acids, respectively. Mota et al. (2016) reported that the average content in leucine and isoleucine is higher in quinoa than in crops like rice, and this could justify the lower differences in 3-methyl-1-butanol and 2-methyl-1-butanol measured between cereals and pseudocereals doughs (except wheat flour).

Related to the control sample, wheat flour dough is located in the negative component of PC1 and PC2 (high proportion of lipid oxidation volatile compounds). When the dough is made, due to the oxygenation during kneading, the content of vitamin E may have reduced, achieving higher lipid oxidation activities. This decrease has been reported to be higher (Alvarez-Jubete et al. 2010; Leenhardt et al. 2006) in wheat bread (47.6%) than rice bread (30.1%) and pseudocereals bread (7.5% in quinoa, 12.3% in buckwheat).

Effect of the flour/starch on the different gluten-free bread crumbs' volatile compounds

In Table 3 the results of the nine fermentation and lipid oxidation compounds found in the crumbs are given in area

Table 3 Fermentation and lipid oxidation volatile compounds identified in all crumbs

	Acetone	Ethyl alcohol	Hexanal	2-Methyl-1-butanol	3-Methyl-1-butanol	1-Pentanol	2-Heptenal	Hexanoic acid	2.4-Decadienal
Wheat starch	8.05 ± 0.63	116.64 ± 14.41	13.52 ± 1.21	0.04 ± 0.005	0.16 ± 0.01	3.28 ± 0.34	0.98 ± 0.09	5.38 ± 0.33	4.12 ± 0.44
Corn starch	5.93 ± 0.44	87.56 ± 10.77	11.54 ± 1.08	0.52 ± 0.060	0.97 ± 0.07	1.63 ± 0.15	2.03 ± 0.18	0.00 ± 0.00	3.14 ± 0.35
Potato starch	7.97 ± 0.71	102.38 ± 12.60	15.58 ± 1.39	0.02 ± 0.003	0.08 ± 0.01	3.20 ± 0.31	1.15 ± 0.13	5.42 ± 0.33	4.34 ± 0.41
Wheat flour	13.74 ± 1.12	211.07 ± 26.12	10.34 ± 0.91	0.03 ± 0.003	0.14 ± 0.01	3.98 ± 0.46	0.98 ± 0.09	2.38 ± 0.17	7.20 ± 0.70
Yellow corn flour	18.15 ± 1.51	330.23 ± 40.82	25.13 ± 2.33	0.03 ± 0.002	0.18 ± 0.01	7.64 ± 0.98	1.82 ± 0.13	5.07 ± 0.32	10.78 ± 1.08
White corn flour	45.18 ± 3.68	283.75 ± 35.17	74.39 ± 6.89	0.06 ± 0.005	0.44 ± 0.04	19.04 ± 2.23	2.21 ± 0.26	1.76 ± 0.15	5.18 ± 0.52
Buckwheat flour	29.58 ± 2.39	82.38 ± 10.17	1.57 ± 0.11	0.03 ± 0.004	0.10 ± 0.01	0.74 ± 0.07	0.18 ± 0.02	1.09 ± 0.08	0.99 ± 0.09
Rice flour	13.33 ± 1.12	177.98 ± 21.93	20.38 ± 1.86	0.01 ± 0.001	0.08 ± 0.01	4.30 ± 0.55	1.51 ± 0.17	5.31 ± 0.35	8.49 ± 0.86
Oat flour	15.63 ± 1.24	296.99 ± 36.70	9.74 ± 0.82	0.18 ± 0.024	0.52 ± 0.05	4.76 ± 0.56	1.03 ± 0.10	1.97 ± 0.14	8.26 ± 0.82
Teff flour	39.35 ± 1.47	263.72 ± 27.62	1.79 ± 0.75	0.20 ± 0.012	0.53 ± 0.03	0.69 ± 0.52	0.26 ± 0.09	1.77 ± 0.16	1.24 ± 0.76
Quinoa flour	56.27 ± 3.26	263.31 ± 32.61	1.43 ± 0.13	0.55 ± 0.025	1.53 ± 0.04	0.67 ± 0.09	0.20 ± 0.02	1.73 ± 0.13	0.71 ± 0.15
Amaranth flour	27.47 ± 4.63	324.05 ± 32.51	9.52 ± 0.15	0.54 ± 0.067	1.13 ± 0.13	4.93 ± 0.08	1.24 ± 0.01	1.45 ± 0.12	3.47 ± 0.08

Results are given in area values ($\times 10^5$). Standard deviations (SD) are given after ± (n = 3)

signal of the target ion to make comparisons. Taking into consideration these compounds, as was previously the case of the doughs, all twelve gluten-free bread crumbs presented the same fermentation and lipid oxidation compounds, but in different concentrations. This was in concordance with those reported by Dall'Asta et al. (2013), who studied the addition of chestnut flour to wheat breads. They found that, although wheat flour was very poor in volatile compounds compared to chestnut flour, wheat bread volatile profile was qualitatively comparable to those obtained for a supplement with chestnut flour, although in different amounts.

With the aim of interpreting the influence of the flour/starch on the generation of volatile compounds in crumbs, another PCA plot was generated (Fig. S2) from the obtained data (peak areas). There is a clear separation between pseudocereals (positive axis) and cereals (negative axis) regarding PC1 of the scores plot, which explains 41.4% of the variability of the original variables. Regarding the loadings plot, lipid oxidation compounds are located in the negative PC1 and fermentation compounds in the positive PC1. Therefore, cereal crumbs presented higher contents in lipid oxidation volatile compounds and pseudocereal crumbs in fermentation volatile compounds (mainly in 3-methyl-1-butanol and 2-methyl-1-butanol).

In general, there is a considerable reduction on the concentration of fermentation volatile compounds in all breads from dough to crumb due to their evaporation during baking. The rate of flavor compounds released depend not only on the volatility of the compound but also on the resistance to mass transfer from the matrix to the air. This resistance to mass transfer has been reported to depend on the macro- and microstructure and texture (Piazza et al. 2008). Therefore, the higher proportion of fermentation compounds measured in quinoa and amaranth crumbs due to a lower evaporation during baking (regarding the data of Tables 2 and 3) may be related with the bread structure. Since pseudocereals flours were wholemeal flours, the bran particles usually puncture and break the gas bubbles decreasing the volume of bread (Hager et al. 2012), leading to more compact breads. In order to understand the losses of volatile compounds during baking regarding the structure, bread volumes were measured and the results are shown in Table 4. In concordance with Hager et al. (2012), pseudocereal breads were much more compact than those of starches, which explain higher releases of ethanol in starches caused by an easier heat penetration. As can be seen in Table 4, the average volume of pseudocereal breads is 3.4 times lower than the average volume of starch breads. Taking into consideration the bread volumes and, therefore, the resistance to mass transfer in compact breads, the tendency of the % losses of ethanol in crumb is logical: starches > rice > oat and corns

Table 4 Percentage of ethanol evaporation from dough to crumb during baking and average bread volumes after baking (n = 2)

Bread	% Evaporation	Volume (cm^3)
Buckwheat flour	88.4	454.0
Potato starch	87.0	1271.0
Wheat starch	86.5	1539.0
Corn starch	82.1	1462.0
Rice flour	80.4	944.5
Oat flour	71.0	406.5
Yellow corn flour	69.1	497.5
White corn flour	66.7	469.0
Teff flour	65.4	425.0
Wheat flour	54.4	603.0
Quinoa flour	56.6	455.5
Amaranth flour	38.4	353.0

flours > teff and pseudocereal flours (Table 4). Thus, the lowest losses of ethanol are present in quinoa and amaranth, justifying their higher content in crumb in fermentation compounds.

The higher content of volatile compounds from lipid oxidation in cereal crumbs, especially in both corn flours, may be related with the balance between the content of lipids (which has not been reported the highest, see Table S1), the lipoxygenase activity and the lipid oxidation inhibitors (vitamin E and flavonoids), as it was explained in 'Effect of the flour/starch on the different gluten-free bread doughs' volatile compounds' section. A possible hypothesis to justify the highest content of lipid oxidation volatile compounds measured in yellow and white corn crumbs and rice crumb (Table 3) may be contributed to their high lipoxygenase activity values, as it has been reported by López-Duarte and Vidal-Quintanar (2009), Maraschin et al. (2008) and Zhang et al. (2009). Wheat and oat flours have also been reported to have lipoxygenase activity (Lampi et al. 2015; Leenhardt et al. 2006), wheat had lower activity than rice (Muñoz et al. 2015), which was in concordance with the result of each crumb in the scores plot (Fig. S2). In fact, oat flour lipoxygenase activity may be much higher, but oat grain may have been heat-treated to inactivate lipoxygenase (Lampi et al. 2015). However, the lipoxygenase activity in quinoa seeds has been reported to be low (Caussette et al. 1997). In addition, as it was explained, the antioxidant activity reported in pseudocereals implies a decrease in lipid oxidation volatile compounds.

It is important to point out that, regarding the PC2, quinoa and amaranth bread crumbs are located in the opposite side of buckwheat. The lower concentration of fermentation volatile compounds in buckwheat should be the cause of the distance between buckwheat and quinoa/amaranth. As it was indicated before, buckwheat presents the highest percentages of losses of fermentation volatile compounds, which could be somewhat related to its bread structure.

Combined results of gluten-free bread doughs and crumbs: the effect of the fermentation and lipid oxidation processes

From dough to crumb there were only differences in the amount of volatile compounds, but no creation nor removal of volatile compounds was observed, related to the SHS-GC/MS analyses. Maillard volatile compounds formed with high temperatures in crust and transferred to crumb, like 2-acetyl-1-pyrroline or 2,5-dimethyl-4-hydroxy-3(2H)-furanone, were not detected by SHS-GC/MS since they are at trace concentration in crumb. These justify the lack of generation of new volatile compounds from dough to crumb. Regarding the Tables 2 and 3, it could be concluded that, in general, doughs showed a higher proportion of fermentation volatile compounds and crumbs a higher proportion of lipid oxidation volatile compounds. This could be explained with the increase in the lipoxygenase action when the yeast activity decreases (Poinot et al. 2009), due to the oxygen necessity of lipoxygenases. Thus, when the rate of fermentation decreases, the lipoxygenase activity increases and, above all, with the elevated temperatures applied during baking the hydroperoxides are decomposed to lipid oxidation volatile compounds.

Towards the selection of the most suitable flour/starch in gluten-free bread aroma quality

Regarding Table 3 (crumb is the final product) and as far as it has been reported in literature, impact aroma compounds with a pleasant fruity perception (positive correlation) have been 3-methyl-1-butanol and 2-methyl-1-butanol and those reported as off-flavors (negative correlation) have been hexanal (grass) and 2,4-decadienal (fatty) (Pico et al. 2015). Quinoa crumb presented the highest content in 3-methyl-1-butanol and 2-methyl-1-butanol and also the lowest content in hexanal and 2,4-decadienal. However, quinoa contains between 0.1 and 5% of saponins (Valencia-Chamorro 2003), which are glycoside compounds that impart a bitter taste (Jancurová et al. 2009), masking the good perception of other compounds. Amaranth showed similar characteristics to quinoa, containing similar content of 3-methyl-1-butanol and 2-methyl-1-butanol but higher content of hexanal and 2,4-decadienal. Moreover, although in smaller amounts than in quinoa, it also contains bitter taste saponins (Oleszek et al. 1999). The improvements of the methods for saponins removal, without significant modifications of nutritive values, were observed (Jancurová et al. 2009). The third crumb containing high amounts of 2-methyl-1-butanol and 3-methyl-1-butanol was corn starch, with 6.5 and 36.9% less

than in quinoa, respectively. The content of hexanal and 2,4-decadienal was almost 10 times and 5 times higher, respectively. However, it does not contain saponins, avoiding the bitter taste. Therefore, corn starch could to be a good option as a base of gluten-free bread in relation to its aroma quality. However, quinoa has been reported as containing high nutritional values in terms of protein, lipids, carbohydrates, vitamins, minerals and fiber (Alvarez-Jubete et al. 2010; Caussette et al. 1997; Hirose et al. 2010; Jancurová et al. 2009), nutritional values that are going to be lower with corn starch. Therefore, a mixture of them seems to be a suitable option.

Conclusion

Yellow and white corn, rice, oat, teff, buckwheat, amaranth and quinoa flours and wheat, corn and potato starches have been employed to compare the volatile profile of their doughs and crumbs. Volatile compounds from fermentation and lipid oxidation in the dough were similar but vary in concentrations. Higher concentrations of fermentation volatile compounds in the doughs and lipid oxidation volatile compounds in the crumbs were observed. Among the different gluten-free doughs and crumbs, the main volatile compounds were also the same, concluding that the type of flour/starch only affected the volatile compounds' concentration from fermentation and lipid oxidation processes (regarding SHS-GC/MS). Quinoa and amaranth crumbs presented the highest content of 2-methylbutanol and 3-methylbutanol (pleasant fruity aromas) but the lowest content of hexanal and 2,4-decadienal (grass and fatty off-flavors, respectively), which would indicated option to improve the gluten-free bread aroma, although their saponins imparted bitter taste. Corn starch was the next with higher content in 2-methylbutanol and 3-methylbutanol but lower content in hexanal and 2,4-decadienal in crumb, but the nutrition values were lower than in pseudocereals. Therefore, the proper mixture of quinoa flour with corn starch seems to be a suitable alternative towards an improved aroma in gluten-free breads.

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