Impact of protein metabolic conversion and volatile derivatives on gluten-free muffins made with quinoa sourdough

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ABSTRACT
A quinoa sourdough inoculated with Lactobacillus plantarum ATCC 8014 vs. a spontaneous sourdough were used to assess the changes in amino acid content released by proteolysis and the volatile derivatives formation in gluten-free muffins. Considerable increases, more than double, in leucine, isoleucine, histidine, lysine, γ-aminobutyric acid were recorded in gluten-free muffins obtained with the inoculated quinoa sourdough fermented for 24 h. Moreover, important markers of aroma compounds like 3-methylbutanal, 2-methylbutanal, 2,3-pentanedione, limonene were found in high and statistically different concentrations when the inoculated sourdough was used. The capacity of these volatiles to transfer pleasant taste and flavor (fruity, citrus, chocolate notes) was correlated with the results of the sensory analysis, clearly highlighting the high consumers’ acceptability for gluten-free muffins obtained with inoculated sourdough. Quinoa fermentation by lactic acid bacteria could be a new alternative to produce gluten-free products enriched in amino acids and with good sensory features.

1. Introduction
Celiac disorder involves the elimination of the gluten and the use of non-wheat cereals in food formulation that could affect the sensorial and textural qualities of the final baked products. Celiac disease is an autoimmune disease caused by ingestion of gluten, a protein found in wheat, barley and rye. The gluten consumed by a celiac person triggers the autoimmune response which is responsible of the damages of the small intestinal villi, preventing proper absorption of vitamins and minerals and exposing the persons at risk for nutritional deficiencies, gastrointestinal cancers, and other autoimmune diseases (Copelton & Valle, 2009).

As supported by a wide number of studies, a few meeting technology challenges and technological approaches could be used to improve the functional and textural quality of gluten-free products such as the addition of quinoa flour, rice flour hydrothermal treatment, fermentation of gluten-free flour by lactic acid bacteria (LAB), sourdough fermentation by LAB (Bourekoua, Benatallah, Zidoune, & Rosell, 2016; Lorusso et al., 2017; Naqash, Gani, Gani, & Masoodi, 2017). LAB represents an inexpensive and safe way to improve sensorial and shelf-life characteristics of the food products (Slapkauskaite, Sekmokiene, Kabasinskiene, Juodeikiene, & Sarkinas, 2016). Because of the consumers’ demands to have healthy, tasty and aromatic leaved baked goods, the renaissance of non-wheat cereals fermentation is a new technological necessity. In addition, as the scientific papers reported that rice, maize, millet, oat, cassava, quinoa, amaranth and buckwheat sourdoughs are non-conventional substrates that can support microbial LAB growth...
2. Materials and methods

2.1. Materials

Quinoa flour, rice flour, eggs, oatmeal, buckwheat flour, coconut butter, corn starch, baking powder, maple syrup were purchased from specialized stores in Romania. Inulin was purchased from Sensus, Netherlands. Lactobacillus plantarum ATCC 8014 (Lp) was purchased from Microbiologics (Minnesota, USA). All analytical reagents and chemicals were purchased from Sigma Aldrich (Louis, MO, USA) and Fluka (Germany).

2.2. Chemical composition

Quinoa flour had the same proximate composition values as reported in a previous work (Chiş et al., 2018) and it is shown in Table 1.

The rice flour, quinoa flour, buckwheat flour, oatmeal, inulin characteristics like moisture, ash, protein (total nitrogen × 5.7) and lipids were determined according to AACC approved methods 45–15 A, 08–01, 46–11 A, 30–10.01, respectively (AACC – American Association of Cereal Chemists, 2010), as showed in Table 1. Total carbohydrates were calculated as the difference 100 – (moisture + ash + proteins + lipids), according to (Rizzello et al., 2017).

2.3. Sourdough preparation

In order to assess the Lp capability to produce sourdough with optimal functional properties, two types of sourdough were prepared, according to (Chiş et al., 2018), as follows: QP-obtained fermenting quinoa flour with Lp and QQ-obtained through a spontaneous fermentation of quinoa flour. The doughs yield (DY) was 200 (DY = dough mass/quinoa flour mass x100) and both sourdoughs were fermented in the same conditions: 37°C, during 24 h. In the QP sourdough, a strain inoculum of log 3.2 CFU LAB/g dough was added. Samples QQ and OP were collected at different fermentation times: 0, 12, 24 h and used like an ingredient in muffins preparation.

2.4. Hydrothermal treatment of rice (oryza sativa) flour

The hydrothermal treatment was made according to (Boureouka et al., 2016), by suspending the rice flour in water at the ratio of 1/5 (w/w). The slurry was heated 7 min, until the temperature of 65°C was reached, cooled down and stored for 24 h at 4°C.

2.5. Batter preparation

Table 2 displays the raw materials for the formulation of batters. The batters were prepared in a mixer (KitchenAid® Precise Heat Mixing Bowl., Ohio), by incorporating firstly rice treated flour, inulin, oatmeal, buckwheat flour, quinoa sourdough and

| Parameters Proximate composition* (%) | Quinoa flour | Rice flour | Buckwheat flour | Oatmeal | Inulin |
|---------------------------------------|-------------|------------|----------------|----------|-------|
| Moisture                              | 10.6 ± 0.6  | 10.4 ± 0.3 | 12.3 ± 0.4     | 10.0 ± 0.3 | 3.1 ± 0.1 |
| Proteins                              | 33.1 ± 0.3  | 8.5 ± 0.3  | 11.7 ± 0.2     | 12.8 ± 0.2 | 6.5 ± 0.2 |
| Carbohydrates                         | 68.2 ± 1.4  | 78 ± 1.0   | 70 ± 0.6       | 12 ± 1.4  | 97 ± 0.1 |
| Ash                                   | 2.3 ± 0.2   | 0.9 ± 0.2  | 2.1 ± 0.4      | 2.8 ± 0.2  | 0.5 ± 0.2 |

*Mean values of three different determinations followed by standard deviation.
* Valores medios de tres pruebas diferentes seguidas de la desviación estándar.

Table 1. Proximate composition of rice, quinoa, buckwheat flours, oatmeal and inulin used in gluten-free muffins formulation.

Table 1. Composición aproximada de arroz, quinua, harinas de trigo sarra- cenno, avena e inulina utilizados en la formulación de panecillos sin gluten.
Table 2. Formulations for gluten-free muffins with sourdough fermented with *L. plantarum* ATCC 8014 (QP) and with spontaneous fermented sourdough (QQ); QPPF- gluten-free muffins obtained with sourdough inoculated with *L. plantarum* ATCC 8014; QPPF- panecillos sin gluten obtenidos con masa madre espontánea.

| Ingredients (%) | QPPF | QPPF |
|----------------|------|------|
| Treated rice flour | 32.50 | 32.50 |
| QQ | 15.00 | - |
| Inulin | 8.00 | 8.00 |
| Oatmeal | 10.00 | 10.00 |
| Corn starch | 7.00 | 7.00 |
| Eggs | 8.00 | 8.00 |
| Baking power | 1.50 | 1.50 |
| Coconut butter | 5.00 | 5.00 |
| Buckwheat flour | 8.00 | 8.00 |
| Maple syrup | 5.00 | 5.00 |
| Total | 100.00 | 100.00 |

The egg yolk was mixed for 2 min at medium speed. The egg white was whipped in a dough mixer (KitchenAid® Precise Heat Mixing Bowl, Ohio) for 2 min at high speed and with the maple syrup was slowly incorporated in the first mixture. After that, the egg white was incorporated in the batters at medium speed. Specific baking trays were used; each muffins cup was filled with 50 g of batter. The muffins were baked in a preheated electric conventional Zanollı oven, at 200°C for 10 min and further at 180°C for 6 min. The baked muffins were left to cool at room temperature for 1 h and packed in vacuum bags at 850 mbar (Multivac C 200, Multivac, Wolfertschwenden, Germany).

2.6. Determination of peptide from quinoa sourdough using SDS-PAGE

2.6.1. Soluble proteins
In order to determine the soluble protein amount the Bradford method was used: the samples were centrifuged at 10,000 × g, 10 min, 4°C (Eppendorf 5804, Germany); and the soluble protein were analysed using bovine serum albumin (Sigma-Aldrich, St. Louis, MO, USA) as the standard. The concentration was determined spectrophotometrically (595 nm) using a nanodrop reader (ND1000 Spectrophotometer) and commercial Bradford solution (Bio-Rad Laboratories, Hercules, CA).

2.6.2. Hydrolysis of proteins by SDS-PAGE
To monitor the hydrolysis quinoa protein extracts with or without Lp, SDS-PAGE – sodium dodecyl sulfate–polyacrylamide gel electrophoresis was used, according to the methods (Dallagnol et al., 2013; Schagger & Von Jagow, 1987; Vilcacundo, Miralles, Carrillo, & Hernández-Ledesma, 2018).

2.8. Volatile derivatives determination by ITEX/GS-MS
The extraction of volatile compounds was performed using the in-tube extraction technique (ITEX), according to (Socaci et al., 2014) with some modifications. Thus, using a CombiPAL AOC-5000 autosampler, 5 g of sample (respectively, 3 g in case of muffin samples) were introduced in a 20 ml headspace vial, sealed and incubated for 20 min at 60°C, under continuous agitation. After incubation, the volatile compounds from the gaseous phase from the vial were adsorbed repeatedly (30 strokes) into a porous polymer fiber microtrap (ITEX-2TRAPXTA, Tenax TA 80/100 mesh, ea) and then were thermally desorbed directly into the GC-MS injector.

The separation of volatile compounds was carried out on a GCMS QP-2010 (Shimadzu Scientific Instruments, Kyoto, Japan) model gas chromatograph – mass spectrometer.
The volatile compounds were separated on a Zebron ZB-5 ms capillary column of 30 m × 0.25 mm i.d and 0.25 μm film thickness. The carrier gas was helium 1ml/min and the split ratio 1:5. The program for column oven was set as follows: 35°C (hold for 5 min) to 155°C at 7°C/min to 260°C at 10°C/min and hold for 5 min. The injector, ion-source and interface temperatures were set at 250°C. The MS detection was performed on a quadrupole mass spectrometer operating in full scan (40–450 m/z) electron impact (EI) at ionization energy of 70 eV.

The volatile compounds were tentatively identified by comparing the mass spectra of each chromatographic peak to NIST27 and NIST147 libraries (considering a minimum similarity of 85%) and whenever possible by comparison with retention indices drawn from www.pherobase.com or www.flavornet.org (for columns with a similar stationary phase to the ZB-5ms column).

This technique offers a qualitative assessment of volatile compounds, the relative percentage of each compound being estimated as a fraction of its integrated ion area from total ion chromatograms (TIC) area (100%).

2.9. Sensory analysis

Sensory analysis of muffins was carried out by 47 panellists (25% male and 75% female, mean age: 41 years, range: 20–63 years), according to the method described by (Coda, Rizzello, & Gobbetti, 2010; Goswami, Gupta, Mridula, Sharma, & Tyagi, 2015). The nine point Hedonic scale was in the following sequence: for 1 to 4 the negative sensations, 5 was neither like nor dislike, and to 6–9, positive sensations, 9 meaning extremely like. Each panel should analyse six muffins (two at 0 h fermentation time, two at 12 h fermentation time, and two at 24 h fermentation time), taking into account the texture, flavour, taste, overall acceptability and appearance.

2.10. Data analysis

Data were compared using Duncan multiple comparison test by performing SPSS version 19 software. Significant differences were indicated by different small letters in the rows (for aroma compounds and for amino acids) when p value was lower or equal to 0.005. All samples analyses were made in triplicates.

3. Results and discussion

3.1. Electrophoretic analysis

Proteins are one of the most important components of quinoa chemical composition and its high nutritional value is due to the high content of free amino acids (Valcárcel-Yamani & Caetano, 2012). The main protein from quinoa is chenopodin, an 11S type globulin, which consists of 49 and 57 kDa subunits having each one an acidic and basic chain (Mäkinen et al., 2016). Protein content from quinoa varies from 13.8% to 16.5% and are similar to the casein from milk, being composed of albumins (35%), globulins (37%) and low quantity of prolamins (Navruz-Varli & Sanlier, 2016).

Generally, proteolysis was higher in Lp inoculated sourdough (QP) than in non-inoculated one (QQ), and it was observed a linear relationship between the stain intensity and the protein concentration in each band. From the stain intensity values, it was calculated the relative ratios among the protein bands of a sample. At the beginning of the fermentation, SDS-PAGE analysis of QP showed nine protein bands with different molecular weights, the 15–35 kDa being the most intensive ones. After 24 h of fermentation, QP protein bands were almost degraded, compared with QQ, were the degradation was lower as can be observed on SDS-PAGE patterns (Figure 1). After 24 h of fermentation, the acid and basic fractions of chenopodins from QP slurry were highly degraded. This fact could be related to the capacity of Lp to degrade peptides and to release amino acids through proteinase and peptidase activities, as reported previously by (Coda et al., 2014; Dallagnol et al., 2013). Peptides deriving from food proteins are of increasing interest for health-promoting functional foods, as dietary supplements and pharmaceutical preparation (Sarmadi & Ismail, 2010). Bioactive peptides are specific protein fragments with a positive impact on the body function or condition and may influence the human health (Rizzello et al., 2017).

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Figure 1. Electrophoretic patterns of QQ (quinoa sourdough obtained through spontaneous fermentation) and QP (quinoa sourdough fermented with Lactobacillus plantarum ATCC 8014) at different moments: 0,8,12, 24 h.

Figura 1. Patrones electroforéticos de QQ (masa fermentada de quinua obtenida mediante fermentación espontánea) y QP (masa fermentada de quinua fermentada con Lactobacillus plantarum ATCC 8014) en diferentes momentos: 0, 8, 12, 24 horas.
For a better analysis of the proteolysis the Coefficient of Protein Degradation (CPD) was calculated. It was presumed that addition of Lp will lead to a greater amount of proteolysis fractions with resulting peptides having a very low molecular mass that need employment of high-through MS technique (peptidome analysis). CPD index allowed to make a comparison between QQ and QP samples during fermentation time based on the optical density of the protein band (OD). The proteolysis degree was expressed as a coefficient of protein degradation (CPD) considering the major protein bands identified, OD for each band and reaction time. The major protein bands after density scanning the gel where between 15 and 35 kDa. After 8 h of fermentation, the QP protein bands from 15 to 35 kDa were hydrolysed giving a CPD value of 25%. At 12 h of fermentation, the CPD index reached a value of 45% while at 24 h a 65% CPD index value was achieved, meaning that the protein hydrolysis increased with the fermentation time. By comparing, the spontaneous fermentation at 8, 12, 24 h leads to a value of 15%, 12%, 18%, respectively. The decrease of the pH influence in a positive way the protein degree hydrolysis (express by CPD) leading to an increase amount of amino acids.

3.2. Amino acids’ content

For an easier discussion, the 22 individual amino acids from sourdoughs and muffins have been grouped according to (Kati, Kaisa, & Karin, 2004) in five groups: aliphatic (glycine, alanine, valine, isoleucine, leucine, proline), basic (arginine, ornithine, lysine, histidine), aromatic (phenylalanine, tyrosine, tryptophan), gamma-aminobutyric acid (serine, threonine, proline, gamma-aminobutyric acid), acids (aspartic acid, glutamic acid, asparagine, glutamine).

In the non-inoculated quinoa slurry, at the beginning of the fermentation (0 h), the most abundant amino acids (in g/100g protein) were represented by aliphatic and basic groups, as follows: leucine (1.2 g/100g protein), isoleucine (1.3 g/100g protein), valine (0.9 g/100g protein), histidine (1.09 g/100g protein), lysine (1.2 g/100 g protein), sulfur amino acid (0.48 g/100g protein), cysteine (0.42 g/100g protein), and γ-aminobutyric acid (0.11 g/100g protein). During fermentation, all the grouped amino acids identified in the QQ sourdoughs increased their concentrations, being statistically different to those from QQ sourdough (Figure 2). The QP’ amino acids (g/100g protein) leucine (2.5), isoleucine (2.7), valine (2.1), histidine (2.2), lysine (2.4), asparagine (1.0), tryptophan (0.9), γ-aminobutyric acid (1.83), increased their concentration in the highest extend. Regarding the spontaneous sourdough QQ, during the fermentation, it was noticed a slight increase of the amino acids, probably due to the quinoa flour endogenous enzymes.

The fermentation of quinoa by LAB could contribute to enhancing its nutritional value by releasing small peptides and amino acids, which are better absorbed in the intestine (Dallagnol et al., 2013; Rizzello et al., 2016). The proteolysis of quinoa sourdough is due both to cereal endogenous protease activated by the low pH and to strain-specific intracellular peptides from L. plantarum. These enzymes significantly influence the accumulation of bioactive peptides and amino acids with antioxidant, antihypertensive or cancer preventing activities (Gobbetti, De Angelis, Corsetti, & Di Cagno, 2005; Gänzle, 2014; Gobbetti, Rizzello, Di Cagno, & De Angelis, 2014). An important result of this study is that in the conditions of the experiment, Lp was able to produce γ-aminobutyric acid (GABA) during quinoa sourdough fermentation, allowing the manufacture of a muffins enriched of GABA in a natural way. γ-aminobutyric acid is a four-carbon amino acid well known due to its important role in central nervous system and its positive effects on human immunity, diabetes and blood cholesterol (Villegas, Brown, De Giori, & Hebert, 2016; Ramos-ruiz, Poirot, & Flores-Mosquera, 2018). A possible explanation for the GABA increment during quinoa sourdough fermentation is the conversion of glutamate into GABA via glutamate decarboxylase mechanism (Gobbetti et al., 2018a) by Lp, reaching a final value of 1.83 g/100 g protein. GABA has different important roles in human body like inhibitor neurotransmitter of the central nervous system, antihypertensive, diuretic, prevention of diabetes, according to (Arendt, Moroni, & Zannini, 2011). The capacity of LAB to synthesize GABA during pseudocereals fermentation was confirmed also by (Coda et al., 2010; Gobbetti et al., 2018a).

Concerning the grouped amino acids amount from the final products, the QP PF 24 h had the highest amount, being statistically different (p < .05) from the QQ PF 24 h final product (Figure 3). The amounts of QP PF 24 h amino

Figure 2. Amino acids’ content in QQ (quinoa sourdough obtained through spontaneous fermentation) and QP (quinoa sourdough fermented with Lactobacillus plantarum ATCC 8014) during fermentation. *Different small letters mean the significant difference (p < 0.05) between QQ and QP at different moments.

Figura 2. Contenido de aminoácidos en QQ (masa madre de quinua obtenida mediante fermentación espontánea) y QP (masa madre de quinua fermentada con Lactobacillus plantarum ATCC 8014) durante la fermentación. *Las diferentes letras minúsculas indican la presencia de diferencias significativas (p < 0.05) entre QQ y QP en diferentes momentos.
acids (g/100 g protein) like leucine, isoleucine, histidine, lysine, γ-aminobutyric acid reached the following value, respectively: 0.40, 0.29, 0.68, 0.61, 0.80 compared with the QQ PF 24 h amino acids which did not reached even the ½ of the QP PF 24 h amino acid content.

### 3.3. Volatile derivatives

As reported by a large body of literature, sourdough fermented by LAB could be an important route of volatile compounds’ production (Pétel, Onno, & Prost, 2017). LAB is able to produce flavour compounds during fermentation of gluten-free flours (Nagash et al., 2017). The basis of LAB fermentation is pyruvic acid, which is produced by LAB through glycolysis of monosaccharides. Pyruvic acid led to several aroma compounds like lactic acid, propanol, ethanol, acetic acid, 2,3 – butanedione, 2-butanone or butanol, propanoic acid. Also, lipid oxidation by lipoxigenase enzymes leads to various aroma compounds like aldehydes (octanal, hexanal, nonanal, heptanal), ketones, esters and alcohols and is directly correlated with the initial raw matrix fatty acid composition (Pétel et al., 2017).

The flavour compounds could be divided into two categories: non-volatile compounds produced by LAB and volatile compounds (includes alcohols, aldehydes, ketones, esters and derivatives). The formation of volatile compounds during sourdough production is influenced by several factors as the type of cereal used as substrate and the free amino acids formed through proteolysis (Rehman, Paterson, & Piggott, 2006).

During the quinoa sourdough fermentation, mainly alcohols (1-hexanol, 1-pentanol, 3-methylbutanal), aldehydes (2-methylbutanal, 3-methylbutanal) and ketones (acetophenone) were formed and accumulated, as showed in Table 3. The dynamic of the volatile derivatives in quinoa sourdough is explained by the metabolism of lactic bacteria through the amino acids catabolism. Thus, by deamination, transamination, decarboxylation and side chain modifications, amino acids are transformed in acids, alcohols, aldehydes (Gobbetti et al., 2005). In the case of QP fermentation, from leucine and isoleucine, amino acids are formed 3-methyl-1-butanol and 2-methyl-1-butanol, important markers of fermentation (Gobbetti et al., 2005). Quinoa is also a rich source of volatile derivatives as it could be seen in initial sourdoughs, explaining its good acceptability among consumers (Pico, Hansen, & Petersen, 2017).

The ratio between alcohols: carboxyls is highest in the case of QP than QQ sourdough. Ethanol was produced only in the spontaneous sourdough (QQ) suggesting a heterofermentative metabolism or the presence of wild yeasts. Also, in QQ sourdough a highest amount of acetic acid was produced.

During baking, due to the Maillard reactions and to the influence of QQ and QP sourdoughs, were noticed different amounts of volatile derivatives (Table 4) comparing to the composition of the sourdoughs in the same compounds. In QQ PF 24 h the higher amount of aroma compounds were represented by ethanol, benzoic and acetic acid which gave to the final product undesirable flavours. This is due to the spontaneous fermentation which depends on the different microflora naturally present in the raw material. On the other hand, the use of QP 24 h gave to the final product compounds like 3-methylbutanal, 2-methylbutanal, 2,3-pentanedione, limonene, which gave to the final product a pleasant taste and flavour. According to Pétel et al. (2017) the main compounds formed during baking through Maillard reaction are directly correlated with aminoacids and sugar dough content. The amino acids formed during sourdough fermentation are precursors of iso-alcohols and contribute directly to the product flavor during baking.

The volatile derivatives quantification revealed a wide range of compounds. As we expected quinoa sourdoughs contained the largest number of volatile derivatives than the gluten-free muffins. Apart from the nutritional advantages, during baking, the free amino acids are converted into volatile compounds which are involved in developing typical flavours of the final product. During baking and thanks to Maillard reactions, results volatile compounds which depend on the amount of amino acids and sugars. For example, the content of 3-methylbutanal increase during baking, probably due to leucine content brought by sourdough into muffins which contribute to the Maillard reaction. This result is confirmed also by the literature (Pétel et al., 2017).
Table 3. Comparative composition of volatile compounds, expressed in arbitrary units (a.u.) separated and identified by GC–MS from sourdough fermented with L.plantarum ATCC 8014 (QP) and spontaneous fermented sourdough (QQ) at different sampling times (0 h, 12 h, 24 h).

| Compounds                  | Odor from the Good Scent Company and (Pétel et al., 2017) | QQ 0h | QQ 12h | QQ 24h |
|----------------------------|----------------------------------------------------------|-------|--------|--------|
| Ethanol, 2-(1-methylethoxy)-methyl butyalcohol | Strong Alcohol, Ethereal, Medicinal                     | nd    | 42.52 ± 0.23 | 50 ± 0.1 |
| 1-Hexanol                  | Ethereal, Oil, alcohol, green, Fruity, Sweet, Woody, Floral | 1.93 ± 0.3 | 5.93 ± 0.2 | 14.51 ± 0.6 |
| 1-Pentanol                 | Balsamine, Oil, Sweet, Chemical mint                     | 1.91 ± 0.79 | 0.96 ± 0.14 | 7.79 ± 0.9 |
| 3-Methylbutanol            | Oil, Alcohol, Fruity, Banana, Whiskey, Almond, Sweet     | 0.41 ± 0.37 | 0.92 ± 0.38 | 10.7 ± 0.24 |
| 2.3-Butanediol             | Fruit, Creamy, Butter, Sour, raifort, tropical fruits, green, vegetable | 0.55 ± 0.15 | 14.95 ± 0.22 | 0.96 ± 0.14 |

**Methylbutanal**
Ethereal, Aldehydic, Chocolate, Fatty, Green, Fruity, Aldehydic, Fatty, Green, vegetable

**Balsamine**
Oil, Sweet, Woody, Floral

**Ethanol**
Aldehydic, Fruity, Sweet, Woody, Floral

**Butanoic acid**
Sharp, acetic, chees, butter, fruity, sweet, sour, sweat, Acetogenic, Aldehyde, Fruity, Sweet, Woody, Floral

**β-Caryophyllene**
Terpenoids

**γ-Caryophyllene**
Terpenoids

**4-Pentyl-1-ol**
Fruity, fermented, Corn flakes, Fruity, Fermented, Corn flakes

**Heptanal**
Fruity, Sweet, Roasted Bread, Fruity, Fermented, Corn flakes

**Hexanal**
Fruity, Fresh, Fruity, Sweet

**Acetophenone**
Fruity, Sweet, Woody, Floral

**Pentane**
Terpenoids

**D-Limonene**
Citrus, grass, terpenes, camphor, Flavour profile analysis of the GFM provided comprehensive information on the aroma characteristics of GFM. The descriptors alcoholic and acidic flavour were identified by panellists in GFM made with QQ sourdough at different fermentation times. This result is supported by the volatile derivatives quantification showing relatively high content in ethanol, benzoic and acetic acids during spontaneous fermentation. The final product manufactured with QQ sourdough was characterised by panellists as having the descriptors fruity, fermented flavour. This could be explained by the formation of the desired aldehyde, limonene and ketones.

Muffin aroma profile analysis showed a significant difference between muffins made with QQ and with QQ sourdoughs. This preference of the panellists for the muffins made with QQ could be explained by the different amount of the limonene, 3-methyl-butanol which gave a fruity, citrus and chocolate odour to the final products.

The panellists, analysing the appearance, texture, taste, flavour, overall acceptability considered that the best product is the muffin made with QQ after a fermentation of 24 h, having a total score of 8.2, as shown in Figure 4. Meanwhile, the sample which received the lowest score was QQPF 12 h reaching a value of 6.06, close to the QQPF 24 h with a value of 6.18.
### Table 4. Comparative composition of volatile compounds, expressed in arbitrary units (a.u.) separated and identified by GC–MS from muffins obtained with inoculated (QP) and non-inoculated (QQ) sourdoughs; QPPF- gluten-free muffins obtained with sourdough inoculated with L. plantarum ATCC 8014; QQPF-gluten-free muffins obtained with spontaneous sourdough.

| Compound          | Odor (from the Good Scent Company and Pétel et al., 2017) | QPFF 0H | QPFF 12H | QPPF 0H | QPPF 12H | QQPF 0H | QQPF 12H | QQPF 24H | QQPF 24H |
|-------------------|-----------------------------------------------------------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| **Alcohols**      |                                                            |        |        |        |        |        |        |        |        |        |
| Ethanol           | Strong, alcohol, ethereal, medicinal.                     | nd     | nd     | 5.83 ± 0.2<sup>a</sup> | nd     | 7.98 ± 0.3<sup>b</sup> | nd     |        |        |        |
| Hexanal           | Fresh, green, fatty, aldehydic, grass, leafy, fruity, sweaty, | 4.83 ± 0.3<sup>b</sup> | 4.26 ± 0.4<sup>b</sup> | 6.84 ± 0.52<sup>c</sup> | 1.41 ± 0.26<sup>c</sup> | 1.25 ± 0.2<sup>c</sup> | 5.06 ± 0.5<sup>b</sup> |        |        |
| Benzaldehyde      | Almond, string, sharp, sweet, bitter, cherry.             | 0.45 ± 0.09<sup>a</sup> | 0.49 ± 0.12<sup>a</sup> | 1.16 ± 0.17<sup>a</sup> | 6.33 ± 0.5<sup>c</sup> | 0.39 ± 0.13<sup>a</sup> | 3.96 ± 0.37<sup>b</sup> |        |        |
| 3 – Methylbutanal | Ethereal, aldehydic, chocolate, peach, fatty, sour, roasted bread, fruity, fermented, corn flakes. | 10.45 ± 0.6<sup>a</sup> | 10.67 ± 0.65<sup>a</sup> | 47.18 ± 0.8<sup>b</sup> | 68.54 ± 0.96<sup>b</sup> | 53.27 ± 0.84<sup>b</sup> | 70.34 ± 1.07<sup>c</sup> |        |        |
| 2 Methylbutanal   | Musty, cocoa, coffee, nut, malty, fruity, sweet, roasted. | 5.49 ± 0.27<sup>a</sup> | 5.29 ± 0.2<sup>a</sup> | 22.41 ± 0.7<sup>b</sup> | 21.56 ± 0.6<sup>b</sup> | 22.75 ± 0.7<sup>b</sup> | 9.6 ± 0.57<sup>c</sup> |        |        |
| Nonanal           | Aldehydic, rose, waxy, citrus, orange, floral             | nd     | nd     | 0.91 ± 0.14<sup>a</sup> | 0.32 ± 0.09<sup>b</sup> | 9.89 ± 0.6<sup>b</sup> | 1.21 ± 0.2<sup>c</sup> |        |        |
| **Ketones**       |                                                            |        |        |        |        |        |        |        |        |        |
| 2,3 – Pentanedione | Floral, almond.                                           | 2.45 ± 0.4<sup>a</sup> | 2.67 ± 0.4<sup>a</sup> | 4.78 ± 0.48<sup>b</sup> | 6.83 ± 0.53<sup>c</sup> | 1.17 ± 0.2<sup>c</sup> | 7.89 ± 0.6<sup>c</sup> |        |        |
| Acetophenone      |                                                           | 1.67 ± 0.27<sup>b</sup> | 1.45 ± 0.22<sup>b</sup> | 2.14 ± 0.3<sup>c</sup> | 3.09 ± 0.37<sup>c</sup> | 0.42 ± 0.08<sup>a</sup> | 5.92 ± 0.21<sup>d</sup> |        |        |
| Others            |                                                           |        |        |        |        |        |        |        |        |        |
| Benzoic Acid      | Faint balsam, urine                                       | 0.79 ± 0.12<sup>a</sup> | 0.85 ± 0.17<sup>a</sup> | 5.33 ± 0.48<sup>b</sup> | 0.32 ± 0.07<sup>a</sup> | 6.72 ± 0.52<sup>b</sup> | 0.27 ± 0.05<sup>a</sup> |        |        |
| Limonene          | Citrus, grass, terpene, camphor                           | 0.06 ± 0.02<sup>a</sup> | 0.048 ± 0.02<sup>a</sup> | 0.95 ± 0.03<sup>b</sup> | 1.32 ± 0.14<sup>a</sup> | 0.13 ± 0.03<sup>a</sup> | 1.46 ± 0.18<sup>b</sup> |        |        |
| Acetic acid       | Sharp, acid, vinegar, sour                               | 0.78 ± 0.15<sup>a</sup> | 0.89 ± 0.17<sup>a</sup> | 1.81 ± 0.2<sup>a</sup> | 1.56 ± 0.2<sup>a</sup> | 2.41 ± 0.3<sup>a</sup> | 1.92 ± 0.27c<sup>c</sup> |        |        |
| 2-Pentylfuran     | Fruity, green, earthy, bean, metallic                     | 0.43 ± 0.09<sup>b</sup> | 0.67 ± 0.14<sup>b</sup> | 1.82 ± 0.2<sup>c</sup> | 1.41 ± 0.19<sup>c</sup> | 0.16 ± 0.03<sup>a</sup> | 1.38 ± 0.16<sup>c</sup> |        |        |

*Nd-not detected.
*The data represent the averages of three measurements.
*The different superscripts in a row indicate significant difference within samples (p < 0.05)
*Nd-no detected
*Los datos representan los promedios de tres mediciones.
*Los diferentes superíndices en una fila indican la presencia de diferencias significativas dentro de las muestras (p < 0.05).

Figure 4. Hedonic scores of GFM (gluten-free muffins) obtained with inoculated (QP) and non-inoculated (QQ) sourdoughs.

Our results are supported by the literature which mentioned that sourdough fermentation by LAB could positively influence the aroma profile of the final product (Pétel et al., 2017; Vermeulen, Ganzle, & Vogel, 2006).

4. Conclusion

Nowadays the range of gluten-free baked goods available in stores offers only poor quality and low palatability. The selection of the raw materials, the formulation and the manufacturing technology could act as major factors of quality improvement. This work demonstrated that the fermentation with Lactobacillus plantarum ATCC 8014 of quinoa flour improved the amino acids and volatile compounds of the sourdough. Also, by using the quinoa sourdough as an ingredient for gluten-free muffins it is possible to obtain specific sensory features for the final baked product.

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**Disclosure statement**

The authors mention that they have no conflict of interest.

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