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Effect of fermentation conditions of bread dough on the sensory and nutritional properties of French bread.

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Highlights

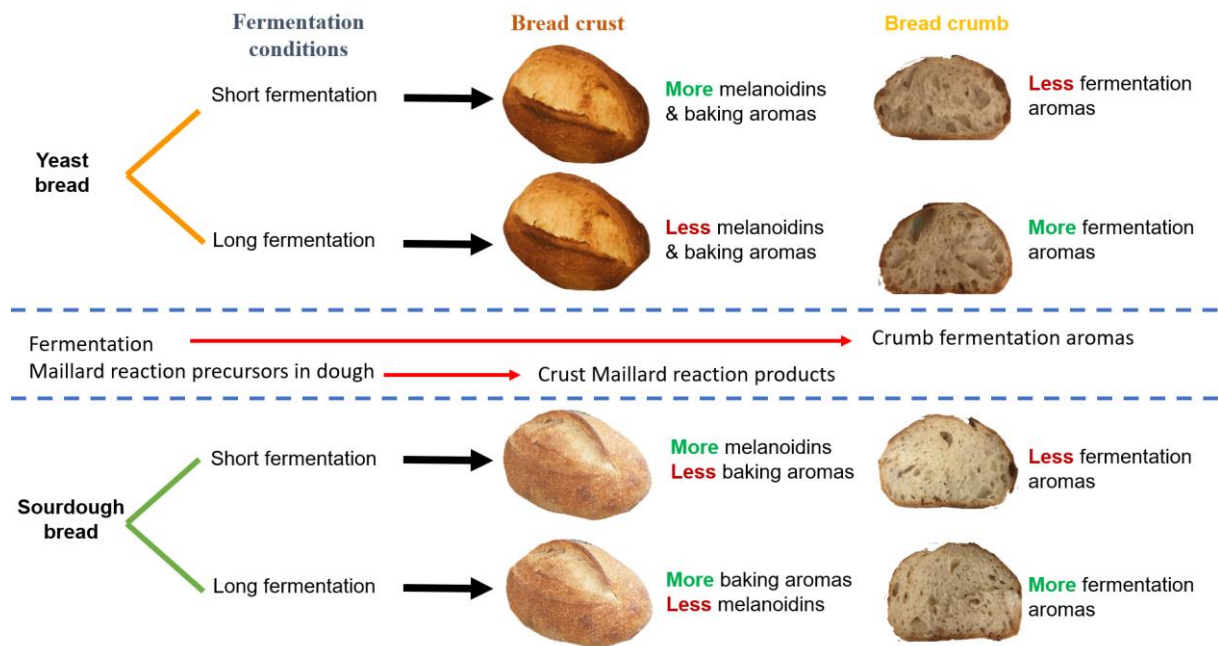
- 1- In sourdough bread dough, long fermentation results in a higher content of reducing sugars than with short-fermentation. Conversely, long fermentation leads to a decrease in reducing sugars in yeast bread dough.
- 2- After baking, the melanoidin content is higher in short-fermented breads.
- 3- After baking, acrylamide formation remains negligible regardless of the type of fermentation.
- 4- Long fermentation has a positive effect on the sensory perception of sourdough bread.

Abstract

The study aimed to evaluate the impact of long and short fermentation on the sensory and nutritional properties of French sourdough and yeast bread. The long fermentation was performed at low temperatures, and is close to “artisanal processes”, while the short fermentation was performed at higher temperatures, and is associated with “intensive processes” used in industries. Reducing sugars and amino compounds, precursors of the Maillard reaction, were analyzed in the doughs, while aromas, acrylamide and melanoidins were quantified in the breads. The study was completed by a sensory analysis performed by a trained panel. The results showed that the content of reducing sugars was highly influenced by the fermentation conditions. On the other hand, proteolysis was limited in both types of bread, especially in yeast bread. In yeast bread, long fermentation favored the generation of aromas from yeast metabolism in the dough, but limited the formation of aromas from the Maillard reaction in the crust. In sourdough bread, long fermentation promoted the Maillard reaction and aromas from the metabolism of lactic acid bacteria and had a positive effect on the sensory perception of the crust and crumb. In both types of bread, long fermentation contributed to the decrease of acrylamide content, even though the overall acrylamide content was low regardless of the type of fermentation.

Keywords: French bread; Maillard reaction; Aromas; Strecker aldehydes; Acrylamide; Melanoidins

Graphical Abstract



1. Introduction

Bread making has been mastered for thousands of years. In France, as in many countries, it takes an important place in the daily diet, with 105 g consumed each day per person [1]. Bread is composed of a mixture of flour, water, salt and leavening agent. The fermentation and baking steps give the bread its final sensory and nutritional characteristics. In France, many types of bread are offered to consumers, but bread fermented with yeast (mostly *Saccharomyces cerevisiae*) remains the most popular and widely consumed. Sourdough bread constitutes a smaller part of the breads purchased by consumers but it is perceived as more qualitative with an extended shelf life [2]. The main difference between these two types of bread is the leavening agent used during fermentation, which has an impact on the sensory and nutritional quality of the bread [3]. Yeasts consume the sugars in the dough to produce ethanol and carbon dioxide through alcoholic fermentation. A small portion of sugars can participate in secondary fermentation reactions that generate aroma compounds [4]. Yeast is often added directly into the flour, along with water and salt to start the fermentation of the bread dough. But yeast can also be used as a pre-ferment in which it ferments wheat flour with water for 4 to 16 hours at room temperature before being added to the bread dough. Another preferment, sourdough is composed of flour and water inoculated with a starter containing lactic acid bacteria (LAB) and yeasts. In addition to yeast-stimulated alcoholic fermentation, sourdough bacteria perform acetic and lactic acid fermentations. The use of sourdough acidifies the bread dough, generates new aromas which improve the crumb aromas, and provide flavor precursors to the dough, such as free amino acids (FAAs), for the Maillard reaction during baking [5–8].

In addition to the diversity of leavening agents, different fermentation conditions can be used to make French bread. In bakeries, artisanal process is often a long fermentation (24 ± 3 h) carried out at low temperature ($6 \pm 2^\circ\text{C}$) [9]. This favors the development of aromas in the dough and is more convenient for the baker because the dough ferments overnight. The bakery industry tends to use straight dough processes with short fermentation times (between 1 and 3 h) at higher temperatures (between 21°C and 35°C), sometimes called intensive process [10] to save time and reduce production costs.

During the fermentation process, the consumption of sugars and proteins present in the wheat flour by leavening agents leads to the formation of alcohols, acids, ketones, and esters in the dough which play a role in the sensory characteristics of the crumb [4]. In yeast bread, it has been shown that long fermentation time at low temperature was positively correlated with high concentrations of aromas in the crumb and the crust [10, 11]. However, the

effect of dough fermentation time on the aroma profile of sourdough bread has been little studied, especially on French bread.

During baking, the Maillard reaction (MR) generates hundreds of compounds in the crust that play an important role in the nutritional and sensory properties of bread [12, 13]. The precursors of the Maillard reaction are the proteins/FAAs and reducing sugars present in the dough and their final concentrations before baking depend largely on the leavening agent used for fermentation [3]. At the intermediate stage of the Maillard reaction, the Strecker degradation converts FAAs into aldehydes that give pleasant malty notes to the crust [4]. The formation of Strecker aldehydes depends on the FAAs available in the dough. Sourdough contains FAAs resulting from the hydrolysis of gluten by bacterial metabolic activity and flour enzymes [14]. Thus, the addition of sourdough to dough formulation provides more flavor precursors for the Strecker aldehydes during baking [6]. In the line of Strecker degradation, acrylamide (AC) is formed during the Maillard reaction. AC is a Maillard reaction product (MRP) that has attracted research attention in recent years because of its deleterious effect on health [15, 16]. It is formed from free asparagine (ASN) and reducing sugars under conditions of low moisture and high temperature (above 120°C). Since fermentation modulates the content of FAAs including ASN in the dough, the formation of AC is highly dependent on the fermentation process [17]. However, we recently showed that traditional French yeast bread was not a significant source of AC in the daily diet compared to other cereal-based products [13] but the impact of fermentation time and temperature on the AC content of French sourdough bread still remains to be elucidated. Melanoidins, formed during the late stages of the Maillard reaction, are brown pigments responsible for the color of the crust. In addition to their role in the sensory quality of bread, melanoidins have been studied for their potential or demonstrated health effects such as their antioxidant properties [18] and anti-inflammatory effect [19]. As they are partly composed of proteins, their content in the bread crust should depend on protein hydrolysis and differ according to the fermentation time.

Considering the fact that the Maillard reaction is strongly dependent on the precursors still available after fermentation, differences between long and short fermentations are expected in terms of fermentation aromas and Strecker aldehydes. The objective of this study was first to evaluate the effect of fermentation conditions on the composition of bread dough (reducing sugars, proteins, free ASN), and then on the aroma profile of breads. We also investigated the effect of fermentation conditions on the nutritional properties of the bread via the AC and melanoidin contents in the crust.

2. Material and methods

2.1. Bread making process

This study was conducted on breads usually consumed in France. The bread making processes were developed according to Myhryold and Migoya [20] and validated by a certified French baker (**Fig. 1**). All doughs had the same hydration rate of 60% (60g of water for 100g of flour). All bread making processes presented in this study have been chosen to obtain similar breads in terms of size and appearance.

2.1.1. Long and short fermented yeast bread

Yeast bread (YB) dough was prepared using wheat flour type T65 (Farine Label Rouge T65, Paulic, Saint-Gérard, France) (61.15%), water (36.7%), salt (1.3%), yeast (0.85%) (Lesaffre, Marcq-en-Baroeul, France) (**Fig. 1A**). Ingredients were kneaded for 10 min at low speed and 4 min at medium speed using a spiral mixer (BV22 Haut, VMI mixing company, Montaigu, France).

1/ For long fermented YB, the dough was fermented for 1 h at 23°C and then for 24 h at 6°C (70% humidity).

2/ For short fermented YB, the dough was fermented for 1 h at 23°C (70% humidity).

All doughs were separated into 250 g pieces and fermented for 30 min at room temperature ($20 \pm 2^\circ\text{C}$). Then, dough pieces were wrought into bread-shape before another fermentation period for 1 h at 23 °C (70% humidity). Breads were baked in a deck oven with steam injection (110 mL) at 250 °C for 25 min. Three batches of 9 breads were made on 3 different days to evaluate the repeatability of the process. After 30 min of cooling at room temperature, the crust and crumb from breads were separated and stored at -20 °C in sealed plastic bags for further analysis. For some analysis, bread crust and crumb were freeze-dried and stored at -20°C.

2.1.2. Long and short fermented sourdough bread

Sourdough bread (SB) dough was prepared using wheat flour type T65 (Farine Label Rouge T65, Paulic, Saint-Gérard, France) (51%), water (26.6%), salt (1.2%), and sourdough (21.2%) as a leavening agent (**Fig. 1B**). Sourdough was prepared the day before by mixing a starter (0.2%) (LV4 starter, LeSaffre, Marcq-en-Baroeul, France) in a mix water/flour (50/50, v/v) and left to rest at 30°C for 24 h (**Fig. 1B**). After the kneading step (as described for YB), dough was fermented for 2 h at 23 °C (70% of humidity), separated in 250 g pieces, re-fermented for 30 min at room temperature ($20 \pm 2^\circ\text{C}$), then wrought in bread shape.

1/ For long-fermented SB, dough pieces were fermented for 24 h at 6°C (70% humidity).

2/ For short-fermented SB, dough pieces were fermented for 2 h at 23°C (70% humidity).

Then, conditions of baking and storage were similar to those described for YB.

2.2. Chemicals, reagents, and standard solutions

Methanol HPLC grade (MeOH), water HPLC grade, acetonitrile HPLC grade (ACN), nonafluoropentanoic acid 97% (NFPA), sodium dodecylsulfate > 99% (SDS), Trizma® base, ethanol 100% (EtOH), acetic acid > 99%, 2-methyl propanol, hydrochloric acid 37% (HCl), ninhydrin reagent, Carrez solutions, trichloroacetic acid >98% (TCA), formic acid >98%, D-glucose, D-Fructose and Maltose standards β -mercaptoethanol were purchased at Sigma-Aldrich (Saint-Quentin Fallavier, France). Internal standard Asparagine (ASN) $^{15}\text{N}_2$ and ASN were from Euriso-top (Saint Aubin, France). Internal standard of ($^{13}\text{C}_3$) acrylamide (AC) was from Restek (Lisses, France). Buffer solution Tris/Glycine/SDS 1X, Mini PROTEAN TGX Gels® and 2X Laemmli buffer solution were provided by BioRad (BioRad, Hercules, USA). The standard L-Glycine was from Megazyme (Bray, Ireland).

2.3. Reducing sugars content

The reducing sugar content was determined in the dough at the beginning of fermentation and after fermentation. The method used was adapted from the one of Epriliati et al [21]. Briefly, reducing sugars were extracted in triplicate from 500 mg of freeze-dried and ground bread dough with 4 mL of 80% (v/v) MeOH in water. Samples were mixed and centrifuged at 20°C during 10 min at 8800 x g. Supernatants were collected and the same steps were repeated once. Extracts were filtered through a 0.45- μm nylon membrane and injected into a HPLC U3000 system (ThermoFisher Scientific, Courtaboeuf, France) coupled to an ELSD LT 90 detector (Sedere, Alfortville, France). The separation was carried out with a water/ACN (25/75 v/v) at a 0.8 mL/min isocratic elution flow using a Prevail Carbohydrate ES column (250 mm x 4.6 mm, 5 μm) and a precolumn (Grace Alltech) at 20°C. Calibration solution containing maltose, glucose and fructose was also injected to calculate reducing sugars content.

2.4. SDS PAGE analysis

Freeze-dried and ground samples (0.028 g of dough) were suspended in tris HCl buffer (50 mM; pH 7.5) containing SDS (1% (w/v)) and β -mercaptoethanol (0.2% (v/v))). Samples were vortexed, placed in ultrasonic bath (Ultrasonic cleaner VWR) for 30 s and shaken at 40 rpm for 40 min (Rotator SB3 Stuart). Samples were centrifuged for 5 min at 10000 x g at room temperature. The supernatants were collected, and diluted (50/50 v/v) in a 2X Laemmli buffer solution pH 6.8 containing 65.8 mM Tris-HCl, 2% SDS, 25% glycerol,

0.01% bromophenol blue. Extracts were heated for 5 min at 95°C in a water bath (Memmert, Schwabach, Germany). SDS PAGE was performed using mini-PROTEAN TGX Gels, 12% polyacrylamide gel (4% stacking gel and 12% resolving gel), 12 wells (BioRad, Hercules, USA). Electrophoresis was performed at 40 mA for 45 min using Tris/Glycine/SDS 1X solution (BioRad, Hercules, USA). Gels were fixed in a trichloroacetic acid 10% for 10 min, stained overnight with Coomassie Brilliant Blue-R250 and unstained using 30% EtOH and 10% acetic acid. Molecular weights were determined thanks to a molecular ladder (Precision Plus Protein™ Unstained Protein Standard, Strep-tagged recombinant, BioRad, Hercules, USA).

2.5. Analysis of volatile compounds

Analysis of volatile compounds was performed according to Jouquand et al. [13]. One gram of ground crust or crumb was mixed with 3 mL of a 2-methyl 1-propanol solution (12.5 ppm) (internal standard). The extraction of volatile compounds was performed using SPME (solid phase micro-extraction). After a sample equilibration time of 15 min at 40 °C, a 2-cm fiber (divinylbenzene-carboxen-polydimethylsiloxane, Sigma-Aldrich) was exposed to the headspace for 1 h at 40 °C, before injection into the injector of the GC/MS with a TriPlus™ 100 LS liquid autosampler (ThermoFisher Scientific, Courtaboeuf, France), in splitless mode, for 2 min at 250 °C. Each extraction was carried out in triplicate. Separation and identification of volatile compounds were carried out using a Trace™ 1600 Serie GC coupled with a ISQ single quadrupole (ThermoFisher Scientific, Courtaboeuf, France). Inlet, ionizing sources, and transfer line temperatures were kept at respectively, 250, 300, and 250 °C. Mass units were monitored from m/z 40 to m/z 250 and ionized at 70 eV. A column of Zebron™ ZB wax plus™ (30m × 0.25 mm × 0.25 μm) (Phenomenex, France.) was used to separate the volatile compounds. Helium was used as carrier gas at a flow rate of 1 mL/min. Identifications were based on matching mass spectra of unknown molecules with those in the NIST mass spectral library or published data.

2.6. Descriptive sensory analysis

Descriptive sensory analysis was conducted at the Institut Polytechnique UniLaSalle (Beauvais, France) by nine well-trained panelists (8 females and 1 male, aged from 25 to 50 years-old), employed by the Institute. Selected attributes were aromatic intensity and bitterness for crust, fermented odor, fermented taste and sweetness for crumb. For SB, additional attributes were added: acidic odor and acidic taste for crumb. For all the sessions, bread was cut into 3-cm slices and served on three-digit numbers coded plates. Samples (YB and SB with both fermentation conditions) were evaluated by panelists in duplicate. Sample presentation was sequential monadic

and followed a balanced design to avoid order and carry-over effects. Panelists were asked to rate the samples for each attribute on a 10-point scale ranging from 0= low intensity to 10= high intensity.

2.7. Colorimetric analysis

Colorimetric measurements were performed 20 times on each bread of each batch, using a portable colorimeter (Model CR-400; Konica Minolta, Japan), with an aperture of 10 mm, illuminant D65. The results were expressed in the international color space CIE $L^* a^* b^*$. The Lightness (L^*) was used to evaluate the level of baking.

2.8. Analysis of water-soluble melanoidins

A semi-quantitative method developed by Helou et al. [22] was used for the quantification of melanoidin content in breads crusts. As the extraction protocol did not consider the insoluble fraction of melanoidins, the water-soluble melanoidins extracted were used as markers of the total melanoidins in bread. Briefly, 500 mg of freeze-dried and ground bread crust were suspended in 4.5 mL of water, mixed for 30 min and centrifuged at 10000 x g for 10 min at 4 °C. Supernatants were collected and filtered through a 0.45- μ m nylon membrane. Extracts were then injected into an HPLC system coupled to a PDA detector and a fluorescence detector (ThermoFisher Scientific, Courtaboeuf, France). The separation was carried out on a phosphate buffer (50 mM) containing 150 mM sodium sulfate (pH 7.0) at a 0.6 mL/min isocratic elution flow using a KW-802.5 (300 \times 8 mm, 5 μ m, 400 Å) column coupled to a KW-G guard column (50 \times 6 mm, 7 μ m) (Shodex, Munich, Germany). The detection was realized at the excitation–emission wavelengths of 350 – 430 nm. An external standard molecular weight calibration curve was used for molecular weight determination using different proteins or peptides (MW 0.5 to 17 kDa). The detection was performed at 280 nm. The group of peaks having a retention time between 17.5 and 27.5 min, which corresponds to the molecular weight of melanoidins, were used for the interpretation.

2.9. Free asparagine content

Free ASN content was measured in bread dough at the beginning and after both type of fermentations using a validated method routinely used in our laboratory. An amount of 200 mg freeze-dried and ground dough was suspended in 7 mL of ultra-pure water. An internal standard ASN $^{15}\text{N}_2$ was added at a concentration corresponding to the calibration range. Samples were vortexed and centrifuged for 10 min at 2500 x g before supernatants collection. The previous steps were repeated twice. The pool of supernatants was clarified with 500 L of each Carrez solution, and centrifuged for 10 min at 2500 x g. Extracts were then filtered through a 0.45- μ m nylon

membrane before analysis, and injected into a HPLC-MS/MS using a Hypercarb (10 mm x 2.1 mm, 5 μ m) column (ThermoFisher Scientific, Courtaboeuf, France). The separation was carried out with NFPA (20mM) / ACN at 200 μ L/min. The percentage of ACN increased from 0 to 10% at 10 min and reached 50% at 12 min. Column oven temperature was set at 25 °C. Tandem MS analyses were performed using a TSQ Quantum Ultra Triple stage quadrupole mass spectrometer (ThermoFisher Scientific, Courtaboeuf, France). Specific transitions m/z 133.0 m/z \rightarrow m/z 87.0 and m/z 135.0 \rightarrow m/z 89.0 were used for the detection and quantification of ASN and $^{15}\text{N}_2$ respectively. ASN concentrations were calculated using a calibration range (ratio between ASN and ASN $^{15}\text{N}_2$).

2.10. Acrylamide content

AC content was measured in the bread crust using a method adapted from the one of Loaëc et al. [23]. Briefly, 1 g of freeze-dried and ground crust was added to 15 mL of $^{13}\text{C}_3$ AC solution as internal standard, mixed during 30 min, and centrifuged for 15 min at 4000 x g at room temperature. An amount of 3.75 mL of supernatant was collected, and the extraction step was repeated once to collect a total of 7.5 mL of supernatant. Carrez solutions I and II were added (750 μ L each), and the resulting solutions were centrifuged for 15 min at 3000 x g. Supernatants were filtered through a 0.45- μ m nylon membrane before analysis by LC-MS/MS. Chromatographic separation was performed according to Loaëc et al. (2014). Samples were injected into a Hypercarb guard column (100 x 2.1 mm, 5 μ ; ThermoFisher Scientific, Courtaboeuf, France), and pre-column with same characteristics. The separation was carried out with formic acid and ACN (1% aq v/v) at a flow rate of 200 μ L/min in isocratic elution mode. Tandem MS analysis was also performed on a TSQ Quantum Ultra triple-stage quadrupole mass spectrometer (ThermoFisher Scientific, Courtaboeuf, France). The detection and quantification of AC and its internal standard were made in SRM mode with the specific transitions m/z 72.0 \rightarrow m/z 55.0 and m/z 75.0 \rightarrow m/z 58.0, respectively. The external standard method was used to determine the concentration of AC in each sample. The limit of detection was estimated at 18 μ g/kg.

2.11. Statistics

All data were expressed in mean \pm SD. Reducing sugars, ASN, and melanoidins data were analyzed by unpaired t-tests with the Graph Pad Prism software (GraphPad Prism version 8.4.3 for Windows, GraphPad Software, San Diego, CA, USA). Sensory analysis data were analyzed by two-ways ANOVA followed by Tukey-Kramer post-

hoc tests with the XLSTAT software (Addinsoft, Paris, France). A value of $P < 0.05$ was considered to be significant.

3. Results and discussion

3.1. Effect of fermentation conditions on the Maillard reaction precursors

The effect of fermentation conditions was evaluated on reducing sugar content (maltose, glucose, fructose) in the dough at the beginning and after the two different fermentation conditions (**Table 1**). In YB dough, maltose content significantly increased of approximately 1.7 times with both types of fermentation compared to the initial content (1654.6 ± 84.2 g/100 g of dry matter). This effect is probably due to the subsequent phenomena of starch hydrolysis leading to an accumulation of maltose [24], and its further hydrolysis by the yeast maltase into glucose [25]. The balance between these two mechanisms occurring simultaneously results in similar amounts of maltose regardless the duration of fermentation. Conversely, the glucose content was divided by two after a short fermentation and by three after a long fermentation ($P < 0.05$). This result confirms that the yeasts preferentially used glucose as a substrate [24]. The fructose content was 1.2 times higher after short fermentation but halved after long fermentation ($P < 0.05$). This increase in fructose content after a short fermentation could be explained by the hydrolysis of sucrose by the yeast invertase [26]. With longer fermentation, the glucose content was no longer sufficient and the yeast began to metabolize fructose as already suggested in the past years [27]. Interestingly, the fermentation temperature does not appear to have much impact on the sugar metabolism by yeasts [28], whereas the optimal temperature of yeast metabolism is above 20°C [29].

In SB dough, the maltose content increased (about 1.5 times) after both types of fermentation, but there was no significant difference between both fermented doughs ($P < 0.05$). Thus, as for YB dough, this is due to starch hydrolysis resulting in maltose accumulation in the dough. Unexpectedly, the maltose content did not decrease after long fermentation even though many sourdough lactobacilli are known to be highly adapted to use maltose as a substrate [30]. The low temperature (6°C) during the long fermentation process could partly explain this low consumption of maltose. Similarly, the glucose content increased after both the fermentation processes, but very slightly after short fermentation and significantly (by 1.5 times) after long fermentation ($P < 0.05$). With this result, we may suggest that, during both the two fermentation processes, the release of glucose by the hydrolysis of

maltose and/or sucrose is compensated by its consumption by the sourdough micro-organisms, explaining the similar levels of glucose [31]. Moreover, glucose seems not to be the main substrate for sourdough lactobacilli, which explains the high levels remaining in the dough after long fermentation.

By contrast to maltose and glucose, the fructose content significantly decreased ($P < 0.05$) in both the fermented doughs, especially after short fermentation. Thus, fructose seemed to be preferred to maltose and glucose as a substrate by sourdough microorganisms. This preference for fructose has already been shown in other studies where the growth of different LAB were tested on different sugar media [32, 33]. During the long fermentation, the low temperature (6°C) could slow down the metabolic activity of the lactobacilli, and thus their consumption of fructose which would explain the higher contents measured in the dough compared to the short fermentation. These first results showed significant variations in reducing sugars content depending on the fermentation conditions used for bread making. Long fermentation led to a higher content of reducing sugars (glucose and fructose) content in the SB dough than in the YB dough. These differences are expected to have an impact on the Maillard reaction pathways during baking, and thus on the sensory and nutritional characteristics of the breads.

The effect of fermentation conditions was also studied on wheat proteins in both breads. Protein extracts from doughs at the beginning and after short and long fermentations were analyzed by SDS PAGE (**Fig. 2**). In both the bread doughs, and according to Osborn classification, the main protein fractions from wheat proteins were visible on the gel. They ranged from high molecular weight glutenin subunit (HMW-GS) (67-100 kDa) and low molecular weight glutenin subunit (LMW-GS) (32-39 kDa), ω -gliadins (39-44 kDa), and α/β - and γ -gliadins (between 28 and 35 kDa), globulin (around 30 kDa) to albumin (5-10 kDa). In both doughs, the wheat protein profile did not change during fermentation regardless of the fermentation time. These results are not surprising since, during yeast fermentation, proteolysis is not promoted [34] because the proteolytic activity of *Saccharomyces cerevisiae* is limited [35], and because the acidic conditions necessary for the activation of flour proteases are not achieved during yeast fermentation [36]. By contrast, several studies have reported proteolysis during sourdough fermentation [7, 37], i.e. during the preparation of this pre-ferment, but few studies have examined the effect of sourdough on proteins once it has been added to the bread dough. Zotta et al. (2006) observed on different protein fractions that sourdough fermentation at 30°C during 6, 12 or 24 h induced protein hydrolysis largely due to the activity of cereal enzymes, which is promoted by the decrease in pH during fermentation. [39] also reported protein hydrolysis during sourdough fermentation at 37°C for 4 or 8 h due to acidic conditions and activity of cereal and microbial enzymes. They and others reported that gliadins were hydrolyzed during sourdough fermentation but not glutenins, the highly polymeric wheat proteins responsible for the strength and elasticity of bread [37, 39]. All

these studies focused on protein hydrolysis during sourdough fermentation. In our study, as the conditions for sourdough fermentation were already set, we sought to evaluate the effect of fermentation time on protein hydrolysis of bread dough formulated with sourdough. These results suggest a limited hydrolysis of proteins during dough fermentation which is not visible on SDS PAGE, regardless of the fermentation time.

Overall, the study of MR precursors (proteins and reducing sugars) in the dough shows that long fermentation led to the decrease of some precursors such as glucose and fructose in YB while it favored their increase in SB. On the other hand, the effect of fermentation conditions was more limited on proteins hydrolysis for the two bread doughs.

3.2. Effect of fermentation conditions on volatile compounds

The overall aroma of bread crust and crumb is composed of hundreds of volatile compounds coming from different reactions that occur during fermentation and baking. In the crumb, volatile compounds are mainly produced by fermentation but some compounds such as aldehydes, ketones, and some alcohols also come from the oxidation of lipids that occurs throughout the bread-making process until baking [4]. Thanks to baking, the MR produces most of the key odorants found in the crust. In this study, 21 compounds were identified in the crumb (**Table 2**) and 23 in the crust (**Table 3**). In YB crumb, long fermentation promoted the formation of ethanol as well as higher molecular weight alcohols such as 3-methylbutanol and phenylethanol via the Ehrlich pathway (secondary fermentation reaction). This result can be explained by the utilization of glucose and fructose by the yeast over a longer period of time and is consistent with the decrease of these substrates observed after a long fermentation (**Table 1**). However, the low contents of glucose and fructose after a long fermentation had an impact on the Maillard reaction during baking, since the furans, ketones and pyrazines identified in the crust were significantly less concentrated than after a short fermentation (**Table 3**). Regarding Strecker aldehydes, the difference between short and long fermentation was less significant. The concentration of 2-methylbutanal and 3-methylbutanal, identified as key odorants in the crust, were similar regardless of the duration of fermentation. This effect is probably due to the similar amounts of FAAs in the dough regardless of fermentation time which could be explained by the limited proteolysis [40].

In SB crumb, the concentrations of alcohols were lower after a long fermentation. The coexistence of yeasts and lactic acid bacteria and their interactions may explain the decrease in alcohol formation in favor of acetic acid formation by lactic acid bacteria. In SB crust, long fermentation resulted in the formation of almost twice as many heterocyclic compounds and four times as many Strecker aldehydes as after short fermentation. This result is a

consequence of the greater availability of reducing sugars after long fermentation. In addition, the high levels of Strecker aldehydes suggest a greater amount of free amino acids present in the dough after long fermentation even though SDS-PAGE analysis did not show significant protein degradation. Thus, this likely increase in FAAs content during long fermentation directed the MR towards the formation of Strecker aldehydes, like 2-methylbutanal and 3-methylbutanal, which have a positive effect on sensory quality of the crust [41].

In summary, in yeast bread, long fermentation favored the generation of aromas from yeast metabolism in the dough, at the expense of aromas from the Maillard reaction generated during baking. Conversely, in sourdough bread, long fermentation promoted the MR and the aromas coming from the metabolic activity of lactic acid bacteria.

3.3. Effect of fermentation conditions on the sensory properties of breads.

The influence of fermentation conditions was evaluated on the sensory characteristics of both breads. A two-way ANOVA (panelists, samples) was applied to test differences among sensory attribute ratings of the two fermentation conditions (**Table 5**). For YB, the statistical evaluation shows that the panelist x samples interaction was not significant ($P > 0.05$) for all attributes indicating that the panelists were consistent in their ratings. However, no significant differences were perceived between the samples. Therefore, the difference between long and short fermentation in terms of aroma profiles presented in **Tables 2 and 3** did not seem to affect the sensory quality of the bread.

For SB, the statistical evaluation shows that there was no significant panelist x sample interaction ($P > 0.05$) (**Table 4B**). This indicates that panelists could discriminate between samples and agreed on perceived differences when they existed. Significant differences between samples were perceived for “aromatic intensity” of the crust and “acidic taste” of the crumb ($P < 0.05$) (**Fig. 3B**). Therefore, a long fermentation seemed to be correlated with a better sensory quality of SB since the aromatic intensity and acidity of the crumb are generally associated with a positive sensory perception of sourdough bread. This result could be explained by the higher concentrations of aldehydes and acids found in the crust and crumb, respectively, after long fermentation (**Tables 2 and 3**).

3.4. Effect of fermentation conditions on melanoidins and acrylamide contents in crust.

Melanoidins are high-molecular-weight (HMW) heterogeneous polymers generated at the last stages of MR. In 2005, Borrelli and Fogliano reported that bread melanoidins had a protein skeleton and consisted of colored compounds bound to the gluten polymer [42]. In our previous study, the evaluation of melanoidins content was

positively correlated with the presence of high molecular weight (HMW) proteins in dough fermented with yeast alone [3]. **Fig. 4** shows the melanoidin contents in the YB and SB crusts as a function of fermentation time. Short fermentation induced higher levels of melanoidins in both types of bread. This result could be explained by a greater availability of high molecular weight proteins after short fermentation even though the results obtained by SDS-PAGE (**Fig. 2**) do not support this. The higher melanoidin level after short fermentation is directly correlated to a darker crust color, for the same baking time (**Fig. 4**). According to these results, we could hypothesize that, a higher melanoidin level in the bread crust could improve the nutritional effect of this bread through an anti-inflammatory and antioxidant effect. It would therefore be interesting to perform further analyses to verify this hypothesis.

Free ASN and AC contents were determined respectively in the dough and crust (**Table 5**). In YB dough, free ASN degradation occurred rapidly due to the activity of yeast extracellular asparaginase [43], resulting in low ASN contents after both short and long fermentations. In SB dough, the free ASN content after short fermentation was not significantly different from that before fermentation, due to the lower yeast activity in the presence of LAB [17]. In contrast, long fermentation significantly decreased the free ASN content, (75% decrease) under the action of asparaginase from the sourdough yeast. These results are in accordance with previous ones who showed a higher consumption of free ASN in yeast bread compared to sourdough bread [17].

A recent study showed that AC levels were lower in sourdough bread compared to yeast bread [44], but the effect of the time and temperature of fermentation on the AC formation had not been studied. In this study, the limit of detection was determined to be at 20 $\mu\text{g}/\text{kg}$ of bread and the limit of quantification was around 52 $\mu\text{g}/\text{kg}$ of bread. Thus, the levels of AC measured in all breads were under the quantification limit (**Table 5**). These results confirm that French bread is not a major contributor to daily AC intake, regardless of fermentation conditions [13]. In YB, no difference was detected between the two fermentation conditions despite of the different ASN contents measured in the doughs. In SB, AC was detected at 32.7 $\mu\text{g}/\text{kg}$ DM after short fermentation while it was not detected after long fermentation. This result can be explained by the lower content of free ASN in the dough after long fermentation.

4. Conclusions

This study aimed to evaluate the impact of fermentation length (short and long fermentation) on the nutritional and sensory characteristics of yeast bread and sourdough bread. The results showed a significant effect of fermentation time on MR precursors content. Long fermentation increased MR precursors in the dough of sourdough bread, while it led to a decrease of these precursors in the dough of yeast bread. These differences had a direct impact on the formation of aromas in the crust and were perceived by a trained panel in sourdough bread. A long fermentation seemed to be correlated with a better sensory quality of sourdough bread. Long fermentation also tended to affect the melanoidin formation. These polymers, being partly composed of high molecular weight proteins, were less concentrated in the crust after long fermentation, resulting in less colored breads than after short fermentation. AC levels were low in all breads regardless of fermentation conditions. Long fermentation was associated with reduced AC levels in the crust of sourdough bread.

In conclusion, long fermentation had a significant effect on the sensory and nutritional qualities of sourdough bread and can be preferred to provide consumers with optimal quality sourdough bread. Its effect was less pronounced on yeast bread and short fermentation resulted in sensory and nutritional qualities that are satisfactory to consumers.

Long-fermented sourdough bread, as found in French bakeries, is therefore interesting in terms of sensory quality but also for its health benefits such as a low glycemic index and a probiotic effect [45]. Other effects such as the impact of bread on the microbiota and the intestinal epithelium are being studied in our laboratory and should allow us to know more about the health effect of bread after digestion.

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Declaration of interest

The authors declare no conflict of interests.

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References

1. FEB (2021) FEB (Quali/Quanti 2021). https://draaf.occitanie.agriculture.gouv.fr/IMG/pdf/3-1_-_QualiQuanti_FEB-Pain2021-pptx_cle0ca1bc.pdf. Accessed 25 Jun 2021
2. Corsetti A, Gobbetti M, De Marco B, et al (2000) Combined Effect of Sourdough Lactic Acid Bacteria and Additives on Bread Firmness and Staling. *J Agric Food Chem* 48:3044–3051. <https://doi.org/10.1021/jf990853e>
3. Troadec R, Nestora S, Niquet-Léridon C, et al (2022) Effect of leavening agent on Maillard reaction and the bifidogenic effect of traditional French bread. *Food Chemistry* 393:133–142. <https://doi.org/10.1016/j.foodchem.2022.133387>
4. Pico J, Bernal J, Gómez M (2015) Wheat bread aroma compounds in crumb and crust: A review. *Food Research International* 75:200–215. <https://doi.org/10.1016/j.foodres.2015.05.051>
5. Hansen A, Hansen B (1996) Flavour of sourdough wheat bread crumb. *Z Lebensm Unters Forch* 202:244–249. <https://doi.org/10.1007/BF01263548>
6. Hansen A, Schieberle P (2005) Generation of aroma compounds during sourdough fermentation: applied and fundamental aspects. *Trends in Food Science & Technology* 16:85–94. <https://doi.org/10.1016/j.tifs.2004.03.007>
7. Katina K, Arendt E, Liukkonen K-H, et al (2005) Potential of sourdough for healthier cereal products. *Trends in Food Science & Technology* 16:104–112. <https://doi.org/10.1016/j.tifs.2004.03.008>
8. Salim-ur-Rehman, Paterson A, Piggott JR (2006) Flavour in sourdough breads: a review. *Trends in Food Science & Technology* 17:557–566. <https://doi.org/10.1016/j.tifs.2006.03.006>
9. Cauvain SP (2007) Dough Retarding and Freezing. In: *Technology of Breadmaking*. Springer US, Boston, MA, pp 175–205
10. Zehentbauer G, Grosch W (1998) Crust Aroma of Baguettes II. Dependence of the Concentrations of Key Odorants on Yeast Level and Dough Processing. *Journal of Cereal Science* 28:93–96. <https://doi.org/10.1006/jcrs.1998.0183>
11. Birch AN, Petersen MA, Hansen ÅS (2013) The aroma profile of wheat bread crumb influenced by yeast concentration and fermentation temperature. *LWT - Food Science and Technology* 50:480–488. <https://doi.org/10.1016/j.lwt.2012.08.019>
12. Purlis E (2010) Browning development in bakery products – A review. *Journal of Food Engineering* 99:239–249. <https://doi.org/10.1016/j.jfoodeng.2010.03.008>
13. Jouquand C, Niquet-Léridon C, Jacolot P, et al (2018) Effects of Maillard Reaction Products on Sensory and Nutritional Qualities of the Traditional French Baguette. *Journal of Food Science* 83:2424–2431. <https://doi.org/10.1111/1750-3841.14331>
14. Gänzle MG (2014) Enzymatic and bacterial conversions during sourdough fermentation. *Food Microbiology* 37:2–10. <https://doi.org/10.1016/j.fm.2013.04.007>
15. EFSA Panel on Contaminants in the Food Chain (CONTAM) (2015) Scientific Opinion on acrylamide in food. *EFSA J* 13:1–26. <https://doi.org/10.2903/j.efsa.2015.4104>
16. IARC (1994) Acrylamide. In: *IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans*. Lyon, France: International Agency for Research on Cancer, pp 389–433
17. Fredriksson H, Tallving J, Rosén J, Åman P (2004) Fermentation Reduces Free Asparagine in Dough and Acrylamide Content in Bread. *Cereal Chemistry Journal* 81:650–653. <https://doi.org/10.1094/CCHEM.2004.81.5.650>

18. Rufián-Henares JA, Morales FJ (2007) Functional properties of melanoidins: In vitro antioxidant, antimicrobial and antihypertensive activities. *Food Research International* 40:995–1002. <https://doi.org/10.1016/j.foodres.2007.05.002>
19. Helou C, Denis S, Spatz M, et al (2015) Insights into bread melanoidins: fate in the upper digestive tract and impact on the gut microbiota using in vitro systems. *Food Funct* 6:3737–3745. <https://doi.org/10.1039/C5FO00836K>
20. Myhrvold N, Migoya FJ (2017) *Modernist bread*. Phaidon, Berlin
21. Epriliati I, Kerven G, D'Arcy B, Gidley MJ (2010) Chromatographic analysis of diverse fruit components using HPLC and UPLC. *Anal Methods* 2:160–166. <https://doi.org/10.1039/c0ay00244e>
22. Helou C, Jacolot P, Niquet-Léridon C, et al (2016) Maillard reaction products in bread: A novel semi-quantitative method for evaluating melanoidins in bread. *Food Chemistry* 190:904–911. <https://doi.org/10.1016/j.foodchem.2015.06.032>
23. Loaëc G, Niquet-Léridon C, Henry N, et al (2014) Effects of variety, agronomic factors, and drying on the amount of free asparagine and crude protein in chicory. Correlation with the acrylamide formation during roasting. *Food Research International* 63:299–305. <https://doi.org/10.1016/j.foodres.2014.03.010>
24. Barber S, Bagueña R, Benedito de Barber C, Martínez-Anaya MA (1991) Evolution of biochemical and rheological characteristics and breadmaking quality during a multistage wheat sour dough process. *Z Lebensm Unters Forch* 192:46–52. <https://doi.org/10.1007/BF01201441>
25. Brondijk H, Konings W, Poolman B (2001) Regulation of maltose transport in *Saccharomyces cerevisiae*. *Archives of Microbiology* 176:96–105. <https://doi.org/10.1007/s002030100300>
26. Nilsson U, Öste R, Jägerstad M (1987) Cereal fructans: Hydrolysis by yeast invertase, in vitro and during fermentation. *Journal of Cereal Science* 6:53–60. [https://doi.org/10.1016/S0733-5210\(87\)80040-1](https://doi.org/10.1016/S0733-5210(87)80040-1)
27. Struyf N, Laurent J, Verspreet J, et al (2017) Substrate-Limited *Saccharomyces cerevisiae* Yeast Strains Allow Control of Fermentation during Bread Making. *J Agric Food Chem* 65:3368–3377. <https://doi.org/10.1021/acs.jafc.7b00313>
28. Liszkowska W, Berłowska J (2021) Yeast Fermentation at Low Temperatures: Adaptation to Changing Environmental Conditions and Formation of Volatile Compounds. *Molecules* 26:1035–1056. <https://doi.org/10.3390/molecules26041035>
29. White J, Munns DJ (1951) Influence of temperature on yeast growth and fermentation. *Journal of the Institute of Brewing* 57:280–284. <https://doi.org/10.1002/j.2050-0416.1951.tb01628.x>
30. Gänzle MG, Vermeulen N, Vogel RF (2007) Carbohydrate, peptide and lipid metabolism of lactic acid bacteria in sourdough. *Food Microbiology* 24:128–138. <https://doi.org/10.1016/j.fm.2006.07.006>
31. Corsetti A, Settanni L (2007) Lactobacilli in sourdough fermentation. *Food Research International* 40:539–558. <https://doi.org/10.1016/j.foodres.2006.11.001>
32. Paramithiotis S, Sofou A, Tsakalidou E, Kalantzopoulos G (2007) Flour carbohydrate catabolism and metabolite production by sourdough lactic acid bacteria. *World J Microbiol Biotechnol* 23:1417–1423. <https://doi.org/10.1007/s11274-007-9384-9>
33. Vrancken G, Rimaux T, De Vuyst L, Leroy F (2008) Kinetic analysis of growth and sugar consumption by *Lactobacillus fermentum* IMDO 130101 reveals adaptation to the acidic sourdough ecosystem. *International Journal of Food Microbiology* 128:58–66. <https://doi.org/10.1016/j.ijfoodmicro.2008.08.001>
34. Diana M, Rafecas M, Quílez J (2014) Free amino acids, acrylamide and biogenic amines in gamma-aminobutyric acid enriched sourdough and commercial breads. *Journal of Cereal Science* 60:639–644. <https://doi.org/10.1016/j.jcs.2014.06.009>

35. Thiele C, Gänzle MG, Vogel RF (2002) Contribution of Sourdough Lactobacilli, Yeast, and Cereal Enzymes to the Generation of Amino Acids in Dough Relevant for Bread Flavor. *Cereal Chemistry Journal* 79:45–51. <https://doi.org/10.1094/CCHEM.2002.79.1.45>
36. Kawamura Y, Yonezawa D (1982) Wheat Flour Proteases and Their Action on Gluten Proteins in Dilute Acetic Acid. *Agricultural and Biological Chemistry* 46:767–773. <https://doi.org/10.1080/00021369.1982.10865134>
37. Gänzle MG, Loponen J, Gobetti M (2008) Proteolysis in sourdough fermentations: mechanisms and potential for improved bread quality. *Trends in Food Science & Technology* 19:513–521. <https://doi.org/10.1016/j.tifs.2008.04.002>
38. Zotta T, Piraino P, Ricciardi A, et al (2006) Proteolysis in Model Sourdough Fermentations. *J Agric Food Chem* 54:2567–2574. <https://doi.org/10.1021/jf052504s>
39. Di Cagno R, De Angelis, Maria M, Lavermicocca P, et al (2002) Proteolysis by sourdough lactic acid bacteria: Effect on wheat flour protein fractions and gliadin peptides involved in human cereal intolerance. *Applied and environmental microbiology* 68:623–633. [https://doi.org/DOI:10.1128/AEM.68.2.623–633.2002](https://doi.org/DOI:10.1128/AEM.68.2.623-633.2002)
40. Benedito de Barber C, Prieto JA, Collar C (1989) Reversed-phase high-performance liquid chromatography analysis of changes in free amino acids during wheat bread dough fermentation. *Cereal Chemistry Journal* 66:283–288
41. Cho IH, Peterson DG (2010) Chemistry of bread aroma: A review. *Food Sci Biotechnol* 19:575–582. <https://doi.org/10.1007/s10068-010-0081-3>
42. Borrelli RC, Fogliano V (2005) Bread crust melanoidins as potential prebiotic ingredients. *Mol Nutr Food Res* 49:673–678. <https://doi.org/10.1002/mnfr.200500011>
43. Dunlop PC, Roon RJ, Even HL (1976) Utilization of D-asparagine by *Saccharomyces cerevisiae*. *J Bacteriol* 125:999–1004. <https://doi.org/10.1128/JB.125.3.999-1004.1976>
44. Nasiri Esfahani B, Kadivar M, Shahedi M, Soleimani-Zad S (2017) Reduction of acrylamide in whole-wheat bread by combining lactobacilli and yeast fermentation. *Food Additives & Contaminants: Part A* 34:1904–1914. <https://doi.org/10.1080/19440049.2017.1378444>
45. Kezer G (2022) Functional Perspective on Sourdough Bread. *Turkish JAF SciTech* 10:1410–1414. <https://doi.org/10.24925/turjaf.v10i8.1410-1414.4860>

Table captions:

Table 1. Reducing sugar contents in doughs at different stages of fermentation.

Table 2. Relative concentrations of volatile compounds identified in the crumb of short or long-fermented breads.

Table 3. Relative concentrations of volatile compounds identified in the crust of short or long-fermented breads.

Table 4. Two-ways analysis of variance of attributes ratings for yeast bread (4A) and sourdough bread (4B).

Table 5. Free asparagine (ASN) content in dough and acrylamide (AC) content in crust of short and long-fermented breads.

Fig. captions:

Fig. 1. Manufacturing diagram of short and long-fermented YB and SB.

Fig. 2. Long and short-fermented YB and SB analyzed by SDS-PAGE. Protein marker is on the left side. The molecular weight of the markers ranges from 5 kDa up to 245 kDa.

Fig. 3: Sensory profile of yeast bread (**3A**) and sourdough bread (**3B**) according to the fermentation conditions.

Fig. 4. Melanoidins in the crust of short-fermented (squares) and long-fermented (circles) YB (white mark) and SB (black mark) crust, according to the lightness (L*) of the crust.

Table 1. Reducing sugars content in the doughs at different stages of fermentation.

Sugars (mg/100 g of DM)	Beginning of fermentation	After short fermentation	After long fermentation
YB			
Maltose	1654.6 ± 84.2 a	2923.3 ± 142.7 b	2814.5 ± 326.6 b
Glucose	217.2 ± 34.3 a	117.3 ± 17.7 b	78.2 ± 7.2 c
Fructose	316.9 ± 33.0 a	390.9 ± 33.0 b	176.6 ± 42.2 c
SB			
Maltose	2245.1 ± 62.7 a	3302.8 ± 76.1 b	3336.0 ± 108.1 b
Glucose	176.7 ± 21.6 a	200.8 ± 19.2 a	267.0 ± 34.4 b
Fructose	170.5 ± 24.9 a	78.0 ± 1.9 b	126.9 ± 27.1 c

YB: yeast bread; SB: Sourdough bread; Different letters in the same row indicate significant differences between groups (p<0.05). Student t-test for unpaired samples (n=9).

Table 2. Relative concentrations of volatile compounds identified in the crumb of short or long-fermented breads.

<i>Compounds</i>	YB		SB		ND = not detected
	<i>Short fermentation</i>	<i>Long fermentation</i>	<i>Short fermentation</i>	<i>Long fermentation</i>	
Aldehydes	Hexanal	0.21 ± 0.08	0.13 ± 0.02	0.11 ± 0.03	0.12 ± 0.01
	Heptanal	0.07 ± 0.02	0.04 ± 0.002	0.03 ± 0.01	0.05 ± 0.01
	2-heptenal	0.03 ± 0.002	0.01 ± 0.003	0.02 ± 0.01	0.02 ± 0.005
	Nonanal	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.001
	2-octenal	0.02 ± 0.002	ND	0.02 ± 0.01	0.02 ± 0.002
	2,4-decadienal	0.03 ± 0.01	0.01 ± 0.001	0.01 ± 0.001	ND
	<i>Trans</i> -2-nonenal	0.04 ± 0.01	0.02 ± 0.001	0.03 ± 0.02	0.03 ± 0.003
	Benzaldehyde	0.11 ± 0.03	0.04 ± 0.01	0.01 ± 0.01	0.06 ± 0.001
Total	0.54	0.27	0.25	0.32	
Alcohols	Ethanol	0.88 ± 0.15	1.67 ± 0.09	0.38 ± 0.03	0.25 ± 0.07
	1-pentanol	0.05 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.05 ± 0.01
	3-methyl-1-butanol	1.07 ± 0.07	2.28 ± 0.16	0.38 ± 0.03	0.23 ± 0.09
	1-hexanol	0.16 ± 0.05	0.14 ± 0.01	0.19 ± 0.06	0.07 ± 0.03
	1-octen-3-ol	0.03 ± 0.002	0.02 ± 0.003	0.02 ± 0.01	0.02 ± 0.003
	Benzyl alcohol	0.02 ± 0.01	0.01 ± 0.001	0.01 ± 0.002	0.03 ± 0.01
	Phenylethanol	0.25 ± 0.07	0.37 ± 0.08	0.42 ± 0.01	0.30 ± 0.02
Total	2.46	4.52	1.43	0.95	
Acids	Lactic acid	ND	ND	0.02 ± 0.01	0.05 ± 0.01
	Acetic acid	0.06 ± 0.01	0.12 ± 0.03	0.44 ± 0.24	0.97 ± 0.16
	Hexanoic acid	0.06 ± 0.01	0.02 ± 0.002	0.01 ± 0.001	0.06 ± 0.01
	Octanoic acid	0.04 ± 0.02	0.01 ± 0.001	0.05 ± 0.03	0.04 ± 0.02
	Nonanoic acid	0.07 ± 0.05	0.01 ± 0.002	0.17 ± 0.05	0.17 ± 0.01
Total	0.23	0.16	0.69	1.29	
Esters	Ethyl octanoate	0.01 ± 0.001	0.01 ± 0.001	0.05 ± 0.03	0.04 ± 0.02
Total	0.01	0.01	0.05	0.04	

Table 3. Relative concentrations of volatile compounds identified in the crust of short and long fermented breads.

<i>Compounds</i>	YB		SB		
	<i>Short fermentation</i>	<i>Long fermentation</i>	<i>Short fermentation</i>	<i>Long fermentation</i>	
Aldehydes	2-Methylpropanal	0.20 ± 0.02	ND	0.22± 0.06	0.92 ± 0.21
	2-Methylbutanal	0.39 ± 0.01	0.38 ± 0.16	1.00 ± 0.27	2.93 ± 0.53
	3-Methylbutanal	0.37 ± 0.03	0.36 ± 0.12	1.82 ± 0.40	5.08 ± 0.38
	Benzaldehyde	0.28 ± 0.02	0.09 ± 0.02	0.04 ± 0.03	0.36 ± 0.09
	Phenylacetaldehyde	ND	ND	0.13 ± 0.08	0.25± 0.05
Total	1.04	0.83	2.99	8.62	
	3-Methylfuran	0.20 ± 0.03	ND	0.07± 0.03	0.20± 0.09

Furans	2-Pentylfuran	0.05 ± 0.003	0.21± 0.07	0.07 ± 0.01	0.70 ± 0.17
	2-(Ethoxymethyl)-furan	0.27 ± 0.05	0.32 ± 0.21	ND	0.14± 0.06
	Furfural	0.43 ± 0.07	0.34 ± 0.12	1.24 ± 0.25	1.06 ± 0.16
	Furfuryl acetate	0.12 ± 0.03	0.03 ± 0.01	0.04 ± 0.001	0.05 ± 0.03
	2-Acetylfuran	0.34 ± 0.09	0.06 ± 0.01	0.17 ± 0.04	0.36 ± 0.14
	5-Methyl-2-furancarboxaldehyde	0.35 ± 0.04	0.05 ± 0.02	0.16 ± 0.07	0.21± 0.13
	2,2'-methylenebis-furan	0.16 ± 0.05	0.26 ± 0.01	ND	ND
	Isomaltol	0.04 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.10± 0.07
	2-Furanmethanol	3.30 ± 0.09	1.24 ± 0.4	0.84 ± 0.20	1.37 ± 0.31
	Maltol	0.37 ± 0.06	0.13± 0.05	0.11 ± 0.03	0.22± 0.08
	5-Hydroxymethylfurfural	0.02 ± 0.01	0.01± 0.007	0.01 ± 0.01	0.02 ± 0.003
Total	5.65	2.67	2.74	4.43	
Ketones	2,3-pentanedione	0.13 ± 0.05	0.04 ± 0.001	0.08 ± 0.02	0.15 ± 0.07
	1-Hydroxy-2-propanone	0.13 ± 0.05	0.02± 0.01	0.03 ± 0.001	0.05± 0.02
	Total	0.26	0.06	0.11	0.2
Pyrazines	Methylpyrazine	0.17 ± 0.06	0.03 ± 0.003	0.07 ± 0.04	ND
	Ethylpyrazine	0.09 ± 0.03	0.01 ± 0.001	0.03 ± 0.01	0.06 ± 0.02
	Total	0.26	0.04	0.1	0.06
Pyrroles	1-(2-furanylmethyl)-1H-pyrrole	0.07 ± 0.02	0.03± 0.01	0.04 ± 0.01	ND
	2-Acetylpyrrole	0.09 ± 0.001	0.03 ± 0.01	0.02 ± 0.002	0.06 ± 0.02
	Total	0.16	0.06	0.06	0.06

ND = not detected

Table 4A: Two-ways analysis of variance of attributes ratings for yeast bread.

	Sensory attributes	Sample (X) F-value	Panelists (Y)	X x Y
Crust	Bitterness	0.56 ns	2.74 ns	1.10 ns
	Aromatic intensity	0.65 ns	0.51 ns	0.46 ns
Crumb	Fermented odor	0.01 ns	1.83 ns	0.40 ns
	Sweet taste	0.01 ns	2.65 ns	0.61 ns
	Fermented taste	1.07 ns	1.59 ns	1.07 ns

ns: not significant

Table 4B: Two-ways analysis of variance of attribute ratings for sourdough bread.

Sensory attributes		Sample (X) F-value	Panelists (Y)	X x Y	*: p<0.05; **: p<0.01; ns: not significant
Crust	Bitterness	0.02 ns	5.23 **	1.11 ns	
	Aromatic intensity	5.34 *	1.81 ns	0.35 ns	
Crumb	Fermented odor	0.18 ns	1.34 ns	1.85 ns	
	Acidic odor	0.33 ns	0.85 ns	1.13 ns	
	Sweet taste	1.27 ns	1.05 ns	0.22 ns	
	Fermented taste	1.27 ns	0.26 ns	0.84 ns	
	Acidic taste	6.33 *	2.79 *	0.68 ns	

Table 5: Free asparagine (ASN) content in dough and acrylamide (AC) content in crust of short and long-fermented breads.

		Beginning of fermentation	After short fermentation	After long fermentation
Free ASN in dough (mg/100 g of DM)	YB	122.2 ± 20.1 a	21.5 ± 4.4 b	9.0 ± 2.7 b
	SB	94.1 ± 10.6 a	95.6 ± 8.4 a	23.4 ± 9.2 b
AC in crust (µg/kg of DM)	YB	-	24.6 ± 6.1 a	21.1 ± 4.6 a
	SB	-	32.7 ± 7.4 a	19.5 ± 4.2 b

Different letters in the same row indicate significant differences between groups (p<0.05). Student t-test for unpaired samples (n=9).

