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Comparison of the antifungal effect of undissociated lactic and acetic acid in sourdough bread and in chemically acidified wheat bread

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ABSTRACT

Sourdough is a very interesting natural preservation system to prolong mould free shelf-life of bread. Numerous studies have reported that the antifungal activity of sourdough is mainly correlated with the presence of lactic (LA) and acetic acid (AA), but very few information is available on the effect of undissociated acid concentrations in the aqueous phase of bread (C_{HA}; mmole/L). This study was conducted to provide additional information about the mode of action of the acids in sourdough bread, enabling a better shelf-life prediction. This study was divided into two parts. In part 1, three industrial biological sourdoughs were characterized (dough yield, pH, a_w, fermentation quotient, microbiota). During 7 weeks, a shelf-life test with natural flora was conducted with daily checks of visible mould growth (21 °C). In part 2, the effect of the acids present in the antifungal active sourdough breads was validated in chemically acidified wheat breads. Complete growth inhibition was observed in full-baked sourdough bread (30 g/100 g dough) containing *Lactobacillus sanfranciscensis* and *Saccharomyces cerevisiae* as dominant sourdough micro-organisms, whereas in control bread the shelf-life was limited to 4.4 – 9.2 days. These full-baked sourdough breads contained 36 mmole undissociated LA/L and 220 mmole undissociated AA/L. The data were used to make General Linear Regression models for shelf-life prediction and resulted in a fit of $R^2 = 0.79$ when expressing the shelf-life in function of $C_{HA,LA}$ and $C_{HA,AA}$. In acidified breads, the role of lactic acid was not significant and only impacted shelf-life indirectly through acidification. No difference between antifungal activity of sourdough breads and chemically acidified bread with comparable $C_{HA,AA}$ concentrations was observed. Shelf-life increased when 150 – 200 mmole undissociated AA/L aqueous phase in bread was present. To conclude, this study showed the importance of the undissociated acid fraction of acetic acid in relation to bread shelf-life, together with bread pH and moisture content.

Keywords: sourdough; acetic acid; undissociated acid; shelf-life; bread

1. Introduction

Mould spoilage caused by species of *Penicillium* spp., *Aspergillus* spp., *Eurotium* spp., *Wallemia* spp., *Fusarium* spp. and *Cladosporium* spp. is a serious problem in the bakery industry (Legan, 1993; Vytřasová et al., 2002). Moreover, the food industry is striving towards *clean label* products free of chemical preservatives (Samapundo et al., 2017). As a solution, sourdough can be used as a natural antifungal agent in bread products (Axel et al., 2016; Debonne et al., 2018b). Sourdough is an unique symbiosis of certain hetero- and homofermentative lactic acid bacteria (LAB) with certain yeasts (Gobbetti, 1998). Traditional sourdough (type I) is characterized by low incubation temperatures and continuous backslopping (De Vuyst & Neysens, 2005). In type I sourdough, maltose-positive *Lactobacillus sanfranciscensis*, *L. brevis*, *L. plantarum* and *L. rossiae* are the most frequently isolated LAB, while maltose-negative *Kazachstania exigua* (formerly *Saccharomyces exiguus*) and *Kazachstania humilis* (synonyms *Candida humilis* and *C. milleri*) are the yeasts that are most frequently present (Brandt et al., 2004; De Vuyst et al., 2002; Gobbetti, 1998; Venturi et al., 2012). During sourdough fermentation, acidification is achieved by the production of weak organic acids by heterofermentative LAB (mainly lactic and acetic acid). These two acids are being held responsible for a microbiological shelf-life extension of bread (Axel et al., 2016; Corsetti et al., 1998; Le Lay et al., 2016). Although acidification is often believed to be the main factor extending the microbiological shelf-life, mould spoilage is not directly affected by low pH values like those of sourdough bread (4.8 – 6.0) (Debonne et al., 2018a; Katina et al., 2002). The pH only plays an indirect role on the activation of lactic and acetic acid by increasing its undissociated/protonated acid concentration (C_{HA}) at low pH values. Only the C_{HA} fraction is able to penetrate the cell membrane causing growth inhibition (Dagnas et al., 2015; Eklund, 1985; Gerez et al., 2009; Lambert & Stratford, 1999). Besides the formation of lactic and acetic acid, other antifungal components can be produced such as phenyllactic acid, antifungal cyclic dipeptides and hydroxyl fatty acids (Black et al., 2013; Lavermicocca et al., 2003; Ström et al., 2002). However, these latter

components are rarely to never produced in antifungal concentrations in sourdough bread (Axel et al., 2016; Quattrini et al., 2018).

The aim of this study was two-fold. First, the antifungal effect of three industrial bio-sourdoughs was evaluated in par-baked and full-baked bread. Second, the concentrations of undissociated lactic and acetic acid in sourdough bread were determined and their antifungal activity was validated in chemically acidified breads. The novelty of this study lies within the fact that in literature often pH is set to compare results of antifungal concentrations of lactic and acetic acid (Alcano et al., 2016; Gerez et al., 2010; Peláez et al., 2012; Quattrini et al., 2018). However in already produced food products such as sourdough bread, the pH cannot be altered. Therefore, a better strategy to compare antifungal active concentrations of weak organic acids in-vitro with food products, is to determine its undissociated concentration (C_{HA}) like has been done in this study. Moreover, only the C_{HA} expressed on the aqueous phase of the breads is of interest as the acids are only present and antimicrobial active in the aqueous phase of the food matrix (Šoljić et al., 2018).

2. Materials and methods

2.1. Experimental set-up

This study investigated the potential of sourdough as antifungal agent in bread. Moreover, this study aimed at determining the antifungal effect of organic acids lactic (LA) and acetic acid (AA) in these sourdough breads. This study can be divided into two parts. In Part 1, the antifungal effect of three industrial bio-sourdoughs was investigated. Ripe sourdoughs were provided by Belgian companies and were characterized: pH, a_w, dough yield (DY), fermentation quotient (FQ), microbiota and total number of LAB and yeasts in the sourdoughs. Further, sourdough breads containing 0, 10, 20 or 30 g sourdough/100 g dough were produced; either par-baked or full-baked; packaged under air atmosphere and stored at 21 °C for 7 weeks (49 days) for the follow-up of fungal growth (shelf-life test). Additionally, the concentrations of total acid of LA and AA in bread (C_{TOT}, mmole/kg bread) were determined through

extraction and HPLC analysis. Further, the concentrations showing extended bread shelf-lives were used for setting up baking trials of chemically acidified wheat breads (Part 2). These acidified breads were also subjected to a shelf-life test of 7 weeks. Moreover, the concentrations of undissociated LA and AA expressed on the aqueous phase of bread (C_{HA}, mmole undissociated acid/L aqueous phase in bread) were calculated and compared between the sourdough breads and the chemically acidified bread.

2.2. Sourdough characterization

2.2.1. pH, aw, dough yield and fermentation quotient

Three ripe sourdoughs (A, B and C) were provided by industrial biological bakeries in Belgium. The dough yields (DY) were provided by the companies. Water activity (a_w) and pH of the sourdough were measured using respectively a LabMaster-A_w (Novasina) and a portable pH meter (model HI 83141, Hanna Instruments). The fermentation quotient (FQ) (ratio of concentration of LA/AA) was also determined. The method of acid determination is elaborated in section 2.5.

2.2.2. Microbiological analysis of sourdough

The microbial composition of the sourdoughs was determined through metagenomics and standard microbiological plating. For the determination of the number of LAB and yeasts, ten grams of sample was diluted tenfold with sterile peptone saline solution (8.5 g/L NaCl + 1 g/L bacteriological peptone (Oxoid LP0037)). After homogenization in a stomacher bag (Novolab NV A11048), dilution series were made and the appropriate dilutions were pour-plated. The dilution series were plated on De Man-Rogosa-Sharpe agar (MRS, Oxoid CM0361) and Oxytetracycline Glucose Yeast Extract Agar base (OGY, Oxoid CM0545) + OGYE supplement (Oxoid SR0073) for the LAB and yeasts respectively. MRS plates were incubated in an anaerobic jar container (Oxoid) with an Anaerogen sachet (2.5 L, Oxoid), guaranteeing an anaerobic environment, for 3 days at 30 °C. OGY pates were incubated for 3 days at 25 °C (Debonne et al., 2018b).

The microbial composition of the sourdoughs was determined by an external company (Genalyse Partner) through the use of metagenomics. This metagenomics analysis was achieved using a Next

Generation Sequencer (Illumine Miseq). Genalyse Partner performed a direct DNA extraction from the sourdough (dilution 1/10) by using a commercial extraction kit with minor modifications. The DNA was amplified by PCR with universal primers targeting the V1-V3 hyper variable region of the 16S rDNA bacterial gene. The quality of the amplifications was checked by an agarose gel electrophoresis in the presence of positive and negative controls. The DNA concentrations of the different amplicons were measured by the PicoGreen system. The DNA sequencing was performed on the Illumina Miseq Platform of Liège University.

2.3. Bread making procedure

2.3.1. Sourdough bread

Sourdough was provided by industrial biological bakeries located in Belgium. Sourdough breads were produced using a single batch of commercial wheat flour (Epi B type 55) supplied by Brabomills NV (Belgium). The flour had the following properties: max 15.5 g moisture /100 g flour, 12 - 13 g protein /100 g flour, max 0.68 g ash /100 g flour. The production of bread dough was similar to the method described in Debonne et al. (2017) and dough was prepared on a flour weight basis. For 100 g flour, 58.6 g water (water absorption was determined by a farinograph (Farinograph-E, Brabender)), 1.5 g table salt, 1 g of instant dry baker's yeast (Algist Bruggeman, Belgium), 0.3 g malt flour and 5 mg ascorbic acid /100 g flour. In case sourdough was added (10, 20 or 30 g SD / 100 g dough), the optimal water absorption was determined for the specific sourdough and concentration, and the recipe was adjusted. The amounts of flour and water added through the sourdough were taken into account to result in an optimal water/flour mixing ratio and a constant flour weight basis of the dough. Additionally, reference breads containing 1500 mg propionic acid (PA) / 100 g dough were prepared (propionic acid was added under the form of calcium propionate due to its increase water solubility). In Europe, 2000 mg PA/ kg (pre-baked) bread is maximally allowed (EU, 2011). Ingredients were mixed in a De Danieli spiral mixer (Verhoest Machinery) for 7 min and dough was placed to rest for 10 min in a proving cabinet (Panimatic)

at 30 °C and 80 to 90 % relative humidity (RH). Dough was divided (30 x 70 g), rounded with a Brabender Rounder and the dough pieces were placed on a perforated, greased plate to prevent sticking. After a fermentation time of 60 min at 30 °C and 80 to 90 % RH, the plate was placed in the oven (MIWE aeromat FB12 (oven type 4.64); external dimensions width: 90 cm; depth = 85 cm; height = 71 cm). Par-baked breads were obtained after a 10 minute two-phase baking process at 150 – 170°C (PB: phase 1: 2' 170 °C, 200 mL steam; phase 2: 8' 150°C). Full-baked breads were subjected to par-baking, followed by another baking step of 10 minutes at 200 – 220 °C (FB: phase 1: 2 ' 220 °C, 200 mL steam; phase 2: 8' 200 °C). This resulted in 15 par-baked breads and 15 full-baked breads for each baking test. The breads were then cooled to room temperature for one hour in the bakery environment which led to a natural post-contamination of the breads with airborne moulds and yeasts. Furthermore, breads were stored in sealed plastic bags (PA/PE/20/70) (PA: polyamide; PE: polyethylene; 90 µm).

Each baking test with combination of sourdough (A, B or C), concentration (0, 10, 20, 30 g/100 g dough or CaP) and baking procedure (PB or FB) was performed in quadruplicate (4 x 15 breads). Every first 15 breads were divided into 5 breads for the air-packed shelf-life test; 5 breads for bread quality characterization and 5 breads for storage for further chemical analysis (test 1). From the second 15 breads, 5 were also used bread quality characterization (test 2). Tests 3 and 4 were equal to respectively 1 and 2. This resulted in a total of 10 replicates for the air-packed shelf-life test and 30 replicates for bread quality analysis.

2.3.2. Chemically acidified bread

Chemically acidified breads were produced in the same way as the sourdough breads. Instead of sourdough, LA and AA were added. Lactic acid (90.2 % AnalaR NORMAPUR, VWR) and acetic acid (glacial, ACS, 99.7+%, VWR) were added to the water phase which was corrected in order to obtain an equal amount of water phase for the dough preparation. The acid concentrations to be tested were determined after having knowledge about the intrinsic acid content of wheat flour bread and the total

acid concentrations in sourdough bread determined via HPLC (C_{TOT} AA: 57 – 100 mmole/kg bread; C_{TOT} LA: 0 – 75 mmole/kg bread).

2.4. Characterization of sourdough breads

One hour after baking, bread weight and bread volume of the sourdough breads were measured by a KERN balance (± 0.01 g) and a Volscan Profiler 600 (Stable Micro Systems). Water activity (a_w) and pH were measured as well (n = 4). Moisture content of bread crumb and crust was determined by the AACCI Method 44-15.02 whereby moisture content is defined as loss in weight of a sample when heated under specified conditions (n = 3).

2.5. HPLC determination of lactic and acetic acid

2.5.1. Sample preparation

Breads and sourdough samples were stored at - 20°C before analysis. Samples (10 g) were homogenized in a glass beaker with 60 mL of 4.0 mM H_2SO_4 solution (Sulphuric acid 97 %, AnalaR Normapur, VWR) using an Ultra-Turrax homogenizer (Yellowline DI25 Basic Homogenizer). Further, the bread suspension was transferred to a 100 mL volumetric flask and supplemented with 4.0 mM H_2SO_4. The suspension was centrifuged for 10 minutes at 25000 G. The supernatant was transferred to 1.7 mL micro centrifuge tubes (VWR) and centrifuged for a second time at 25000 G. Further, the supernatant was filtered over a 0.45 µm filter (PTFE-membrane, 13 mm diameter; VWR) directly into 1.5 mL glass vials (VWR). Three samples of each baking tests were analyzed.

2.5.2. Equipment and analytical procedure

The concentrations of LA and AA in the supernatant were determined using an Agilent 1100 HPLC system. Separation was carried out with a Biorad Aminex HPX-87H column (300 x 7.8 mm) (Bio-Rad Laboratories). The mobile phase was 4 mM H_2SO_4 (flow rate of 0.6 mL/min) and the temperature of the column was 35 °C. Samples were measured with UV VIS at wavelength 210 nm. Injection volume was 10 µL. Calibration curves of LA and AA were prepared to calculate the concentration of the compound in the

samples. All sample extracts were prepared in duplicate of which one replicate was spiked with a known concentration of LA and AA to verify the peak at the correct retention times. The results obtained from the HPLC method were expressed in mmole acid/kg bread or sourdough (C_{TOT}).

2.5.3. Concentration of undissociated acid in bread samples

The results obtained from the HPLC analyses expressed in mmole acid/kg bread were recalculated to mmole undissociated acid/L aqueous phase in bread. Organic acids are considered weak acids when they do not fully dissociate in water, but in a pH-dependent manner. The active concentration of the organic acid is defined as the concentration of undissociated acid in the aqueous phase of the medium. This recalculation involved using data of the percentage of dissociation of the acid in function of the measured pH, which could be derived from the Henderson-Hasselbalch equation [1], and the moisture content of bread.

$$pH = pK_a + log_{10}(\frac{[[A^-]}{[HA]})$$

[1]

The *pKa* is equal to -$\log_{10}$(Ka) with Ka the acid dissociation constant. The *pKa* values of LA and AA are respectively 3.86 and 4.75; *[A-]* is the molar concentration of the acid's conjugated base and *[HA]* is the molar concentration of the undissociated acid. From this equation (Eq. 1), the percentage of dissociation ([H+]/[HA]) could be derived and used for determining the concentration of undissociated acid (C_{HA}) together with the total concentration of the acid (*T*) (Eq. 2):

$$C_{HA} = T\ x\ (1 - \frac{\%\ dissociation}{100})$$

[2]

2.6. Shelf-life test of bread

The breads were stored at 21 ± 1 °C and the samples were checked daily during 49 days for the development of visible mould colonies (n = 10 for air-packed breads).

2.7. Statistical analysis

To assess significant differences among samples (e.g. pH of bread), a multiple comparison analysis of samples was performed using *SPSS Statistics 25*. In case the results were normally distributed, either a Tukey test (homoscedasticity) or a Dunnett T3 test was used to describe the means with 95 % confidence (p = 0.05). A Dunn test for multiple comparison was applied, preceded by a non-parametric Kruskal-Wallis 1-way ANOVA, for non-normally distributed data. Assuming there is a linear relationship among the main effects of SD type, baking condition, concentration of LA and AA in bread, pH, a_w, the interaction effects and the mould free shelf-life, a General Linear Univariate Model was developed using the data. Parameters *SD type* and *baking condition* were fixed variables (nominal variables inserted by dummy coding) and *acid concentration*, *pH*, a_w were covariates (scale variables). *Shelf-life* was the dependent variable. The model terms were selected by the backward stepwise procedure, based on their significance (p < 0.05). Model building stopped when no more variables met entry or removal criteria.

3. Results and discussion

3.1. Sourdough characteristics

Three industrial sourdoughs (type A, B and C) were used in this study. They were produced in their own bakery environment, which was separate from the Laboratory of the Research Unit of Cereal and Feed Technology (Ghent University, Belgium) where this study was conducted. SD can be characterized depending on various parameters (Table 1). On the one hand, dough yield (DY) can be used to describe

the consistency of the SD. It expresses the total weight of SD relative to the amount of flour present in the SD. A DY of 200, as is the case for SD type C, means that the SD consists of equal amounts of water and flour. A lower DY of 166.5 and 161 (resp. types A and B) means that there is less water present in the SD than there is flour, resulting in a more solid-like SD. On the other hand, SD can be characterized by the presence and number of specific lactic acid bacteria (LAB) and yeasts (Table 1 and 2). LAB are mainly responsible for acidification of dough, while yeasts are mostly involved in the leavening process and in the production of volatile compounds. *Lactobacillus sanfranciscensis* dominated the LAB populations in SD types A and B, while *Weissella confusa* dominated type C (Table 2). A stable SD microflora consisting of *L. sanfranciscensis* and *Kazachstania humilis* (type A) is characteristic for type I SD (traditional SD) and is the most occurring SD system (Brandt et al., 2004). In some cases of type I SD, baker's yeast (*Saccharomyces cerevisiae*) is added during fermentation for increasing the leavening potential. This is presumably the case in type B SD. In type C SD, *W. confusa* dominated the LAB population (62.2 %). Compared to species of *Lactobacillus*, species belonging to the genera of *Weissella* are much less frequently encountered in SD, although several of these species are present in the cereal kernels and flour or during the early fermentation process (De Vuyst et al., 2009). SD microflora consisting of *W. confusa* and *S. cerevisiae* (type C) and a DY of 200 or higher is characteristic for type II SD (accelerated SD). Under the growth conditions of type II SD, *L. sanfranciscensis* is not competitive enough to dominate the fermentation (De Vuyst & Neysens, 2005).

The pH of the ripe sourdough was 4.9 for type A and 4.0 – 4.1 for types B and C. The ideal ratio of LAB over yeasts is 100/1 (Schnürer & Magnusson, 2005). Type A has a ratio of 40/1, whereas type C has a ratio of 86/1. Type B contained less yeasts (5.3 log/g) and resulted in a ratio of $5*10^3/1$. In types B and C, *S. cerevisiae* is the most dominant yeast. In literature the presence of maltose-positive yeast *S. cerevisiae* in type I sourdough is often reported (Venturi et al., 2012; Vogelmann et al., 2009). However according to De Vuyst et al. (2009), its presence might be mostly ascribed to the use of commercial baker's yeasts

during sourdough fermentation or to its presence in the bakery environment. For example in this study, the high amounts of LAB in type B and C, together with the rather competitive interaction for maltose of maltose-positive LAB and maltose-positive yeast indicates the use of starter cultures or the use of baker's yeast added to the first refreshment to speed up the last leavening step (Garofalo et al., 2008).

Organic acid production during SD fermentation depends to various extents on microbial starter composition and on process parameters such as type of flour (e.g. ash content), DY, fermentation time and temperature and NaCl concentration (Robert et al., 2006; Salovaara & Valjakka, 1987). Maltose is the preferred carbon source for the heterofermentative LAB found in this study. A majority of strains of *L. sanfranciscensis* is unable to utilize fructose as carbon source (Gänzle et al., 2007). However, it is able to use fructose as an alternative electron acceptor favoring their competitive advantage in SD. This results in increased amounts of AA from acetyl-phosphate as ethanol production is no further necessary for NAD+ regeneration (De Vuyst et al., 2009; Röcken et al., 1992). In our study, the presence of *L. sanfranciscensis* in SD (type A and B) resulted in high AA concentrations (89 - 99 mmole/kg SD). DY has also been reported to exert a significant role on AA production (low DY = increased AA production). However altering the availability of the hydrogen acceptor (via fructose or invert sugar supply) is a more effective way of influencing the production of AA (Röcken et al., 1992). In type C SD, concentrations of AA and LA of respectively 39 ± 1 and 53 ± 2 mmole/kg SD were measured. Similar concentrations were reported by Baek et al. (2012) in water-soluble extracts from *W. confusa* - fermented rice dough (resp. 41.6 mM AA and 60.3 mM LA).

3.2. Antifungal effect of sourdough bread

3.2.1. Shelf-life of sourdough bread

After characterization of the sourdoughs, baking trials and shelf-life tests were set up. The variables in this study were concentration and type of SD and par-baked (PB) or full-baked (FB) bread. The a_w values

of the PB and FB bread crumbs in this study were not significantly influenced by type and concentration of SD ($p > 0.05$). They were respectively 0.94 ± 0.01 for PB and 0.91 ± 0.01 for FB bread (n = 20). Looking at the results in Figure 1, the effect of par-baking versus full-baking is clearly visible. Control PB bread without SD has a short mould free shelf-life of 4.4 ± 0.6 days, whereas control FB bread has the tendency to extend visible mould spoilage to 8.3 ± 4.0 days (Table 3). These shelf-life times are in line with previous results reported in Debonne et al. (2018b). In that same study it was observed that at higher baking times and temperatures, the a_w of bread decreases resulting in a longer mould free shelf-life.

During 7 weeks of incubation, only 30 g SD type B/100 g dough was able to completely prevent mould spoilage on FB bread (Table 3). Overall, SD type B showed to have the best preservation potential in FB bread. This SD was characterized by containing the highest total concentration of LA and AA (Table 1). 20 g SD type B/100 g dough was sufficient for significantly increasing the shelf-life of bread from 8.3 ± 4.0 days to 31.2 ± 13.0 days (Table 3) whereas SD types A and C could not extend the shelf-life of FB bread significantly. In PB bread, 20 g SD/100 g dough of all three SDs could significantly increase the shelf-life from 4.4 ± 0.6 days to 12.6 ± 3.8 (type A), 9.0 ± 0.0 (type B) and 8.9 ± 2.1 days (type C). The shelf-life extending effect of SD in PB bread is much less compared to the effect it had on FB bread.

3.2.2. Activity of organic acids in sourdough bread

The concentrations of the acids were determined by HPLC analysis. The total concentrations of the acids expressed in mmole acid/kg bread crust (C_{TOT}) and the recalculated concentrations of the undissociated fraction expressed in mmole undissociated acid (HA)/L aqueous phase in bread crust (C_{HA}) can be found in Table 4. In control bread (PB and FB) 0 mmole lactic acid/kg bread crust and 54 – 60 mmole acetic acid/kg bread crust (C_{TOT}) were measured. The presence of acetic acid in the reference bread is due to the degradation of flour fructans by baker's yeast. *S. cerevisiae* is known for its capability of producing acetic acid under both aerobic and anaerobic/oxygen-limiting conditions. The conversion of glucose to

acetic acid occurs parallel with the reduction of NAD^+ (Uscanga, Delia, & Strehaiano, 2003; Walker & Stewart, 2016).

The C_{HA} of LA/AA in the crust of the SD bread system with complete growth inhibition during 7 weeks was 36/220 (ratio of concentrations expressed in mmole HA/L aqueous phase). This was in FB bread with type B SD (30 g/100 g dough). In FB control bread the acid ratio was 0/15 (shelf-life: 8.3 - 9.2 days). Other significantly increased mould free shelf-lives of FB breads were observed for type C (30 g/100 g: 16.0 days, 9/141), type B (20 g/100 g: 22.9 – 31.2 days, 19/172) and type A (30 g/100 g: 32.4 days , 3/159). In PB control bread the acid ratio was 0/13 (4.4 – 8.4 days). Significant effects on PB bread shelf-life were observed for type A (20 g/100 g: 10 - 12.6 days, 1/35 – 2/38; 30 g/100 g: 14.2 – 19 days, 3/61), type B (20 g/100 g: 9 days, 15/121; 30 g/100 g: 13.7 - 14.8 days, 30/157) and type C (20 and 30 g/100 g dough: 8.9 – 9.0 days, resp. 7/91 and 9/99).

The main goal of this study was to investigate whether LA and/or AA were responsible for a shelf-life increase in sourdough bread and to what extent the concentrations of undissociated acid can predict mould-free shelf-life. Therefore, General Linear Regression models were fitted and significant parameters determining the shelf-life of bread were defined. Two models were developed for the data of the sourdough breads, one with the C_{TOT} concentrations (y_1) and one with C_{HA} concentrations (y_2) (Table 5). Model y_1 shows that the mould free shelf-life of bread is function of the *SD type* (A, B or C), *baking condition* (PB or FB), $C_{TOT, LA}$ (mmole LA/kg bread), $C_{TOT,AA}$ (mmole AA/kg bread) and *pH*. All parameters contributed significantly to the model ($p < 0.05$) with an overall fit of $R^2 = 0.64$. In order to link the antifungal activity of SD bread to the undissociated acid concentrations of LA and AA, the total concentrations of the acids were recalculated to undissociated concentration (C_{HA}, Table 4) and these data were used to make a new model prediction of bread shelf-life (y_2). The parameters taken up in the model were *SD type*, *pH*, a_w and $C_{HA,AA}$ and $C_{HA,LA}$. The fit of the new model with all significant parameters ($p < 0.05$) was $R^2 = 0.79$ (Table 5). In this equation (y_2), the main effect of $C_{HA,LA}$ was not significant.

However, the interaction terms of $C_{HA,LA}$ with $C_{HA,AA}$ and pH were significant and part of the model prediction. It is known that AA has the strongest antifungal activity in bread compared to LA (Quattrini et al., 2018). For example, at normal pH of (sourdough) bread of around 5 only 7 % of LA is undissociated, whereas for AA this is 36 % (Gerez et al., 2009). Nevertheless, LA is important in preservation due to its strong pH lowering effect, resulting in an increase of $C_{HA,AA}$.

3.3. Antifungal activity of organic acids in chemically acidified bread

Potential antifungal concentrations of LA and AA derived from the study with SD breads were selected for the validation experiment in chemically acidified wheat breads. In type B SD bread, ratio 36/220 of undissociated LA and AA (C_{HA}; mmole/L aqueous phase in bread crust) was shown to completely prevent fungal growth during 7 weeks (Tables 3 and 4). This corresponded with C_{TOT} 49 mmole LA/kg bread and 85 mmole AA/kg bread (49/85) (Table 4). Before baking, the relation between acid content in dough and in baked bread was determined (Supplementary data - Figure S1). Based on these data, baking recipes were defined. LA was tested within the range of C_{TOT} 0 – 75 mmole/kg bread and AA within 57 – 100 mmole/kg bread. The combinations of LA and AA can be found in Table 6. Similar to the SD baking trials, PB and FB breads were produced and subjected to a shelf-life test of 7 weeks. After baking, pH and moisture content of the breads were determined to calculate the undissociated acid concentrations of the acids (C_{HA}). This data was used for fitting a third linear regression model (y_3) (Table 5). The shelf-life of chemically acidified wheat breads was function of $C_{HA,\,AA}$ and baking condition (PB or FB) ($R^2 = 0.66$). Like model y_2, the effect of $C_{HA,LA}$ is not part of the model prediction ($p > 0.05$). This result shows that the undissociated acid concentration of acetic acid is the main factor determining bread shelf-life in sourdough breads and chemically acidified wheat breads.

The pH of 0/100 ($C_{TOT,LA}/C_{TOT,AA}$; mmole/kg bread) PB bread was 4.5, meaning that 64 % of AA is present in its undissociated form which corresponds to $C_{HA,AA}$ 188 mmole/L aqueous bread phase in bread crust

(Table 6). In PB bread, conditions with C_{TOT} 100 mmole AA/kg bread and 25, 50 or 75 mmole LA/kg bread all prolonged shelf-life up to 7 weeks (Table 3). LA seems to not influence shelf-life because the maximal shelf-life is reached with all three concentrations of LA. However, LA lowers bread pH to respectively 4.1, 4.1 and 3.8, hereby increasing the concentrations of undissociated AA (resp. 82, 82 and 90 %) ($C_{HA,AA}$ > 240 mmole /L). The C_{HA} of LA in 25/100, 50/100 and 75/100 breads were 27, 54 and 118 mmole/L. In SD bread, the concentration of undissociated acid preventing mould spoilage was 36/220 (pH = 4.3).

3.4. Comparison of antifungal activity in sourdough breads with chemically acidified breads

In order to compare the effect of the organic acids, shelf-life data of both groups of SD breads and chemically acidified breads were clustered based on the undissociated acid concentrations of AA (C_{HA} 0 – 100; 100 – 150; 150 – 200 and > 200 mmole/L) (Figure 2). A shelf-life increase was observed for all breads containing $C_{HA,AA}$ of 150 – 200 mmole/L or higher. There was no difference between antifungal activity of AA in SD bread compared to the effect in chemically acidified bread. At $C_{HA,AA}$ > 200 mmole/L, the effect of AA is the most important inhibiting factor whereas at lower concentrations the effect of baking and a_w can still influence mould free shelf-life. Important to note as well is that the pH of SD bread and chemically acidified wheat bread is very similar when clustering the data based on its $C_{HA,AA}$ content (e.g. in cluster 150 – 200 mmole AA/L, the pH ranged between 4.4 – 4.6 in SD bread and 4.2 in chemically acidified bread). The pH values per $C_{HA,AA}$ range were in following order; [0 – 100]: 5.3 ± 0.4; 5.1 ± 0.3; 5.8 ± 0.0; and 5.6 ± 0.1; [100 - 150]: 4.6 ± 0.2; 4.2 ± 0.2 ; 4.6 ± 0.3 and 4.8 ± 0.2; [150 – 200]: 4.4 ± 0.0; 4.2 ± 0.2; 4.6 ± 0.2 and 4.2 ± 0.2; [> 200]: 4.2 ± 0.1; 4.1 ± 0.2 and 4.1 ± 0.2.

3.5. Quality of sourdough breads

In Table 7, the quality parameters of SD and chemically acidified wheat breads are represented: weight, and volume. A_w and moisture content of PB/FB and crust/crumb of sourdough breads were not significantly different amongst different SD types and concentrations and are therefore not taken up in

the table. The a_w of PB bread crumb and crust were 0.95 ± 0.01 and 0.94 ± 0.01. The values for FB bread crumb and crust were 0.93 ± 0.01 and 0.91 ± 0.01 (n = 50) (measured 2h after baking). Moisture content of PB bread crumb and crust were respectively 44 ± 1 and 34 ± 3; and for FB bread crumb and crust: 43 ± 1 and 29 ± 3 (n = 20). Control PB bread volume was 159 ± 21 mL whereas bread with 10 – 30 g SD type C/100 g dough or higher resulted in a volume of 202 - 243 mL. Based on the lower acid production of type C SD, it is plausible that *W. confusa* is less competitive towards maltose compared to *L. sanfranciscensis*. Moreover, due to the lower concentration of AA, yeast-leavening activity is less effected resulting in a larger bread volume. The pH of all PB SD breads was significantly reduced using 20 g SD/100 g dough. In FB bread, the pH was already reduced at 10 g SD/100 dough because there is less aqueous phase available in which the acids are dissolved when full-baking The lowest pH was obtained in FB SD bread with 30 g type B SD/100 g dough (pH = 4.2 ± 0.1). Evaluation of the chemically acidified wheat breads was also performed (Table 7). Bread volume of the chemically acidified breads was strongly reduced by the organic acids. Volume was more affected by LA (significant reduction between 25 – 50 mmole/kg bread (C_{TOT})) than AA (75 – 100 mmole/kg bread). In this study, it was not the intention to produce qualitative chemically acidified wheat breads. High concentrations of acids were added during bread production in order to define antifungal concentrations of undissociated acid. Moreover, this study had the objective of comparing the antifungal activity of C_{HA} of acetic and lactic acid in sourdough bread with acidified wheat breads in order to exclude other potential preservatives present in sourdough bread. This study showed that fermentation processing is essential to produce sensorial acceptable bread products with antifungal activity.

4. Conclusion

This study showed the importance of the undissociated acid fractions of lactic and acetic acid in sourdough bread. The prediction of mould free shelf-life of sourdough bread was enhanced from 64% to 79% by expressing the shelf-life in function of undissociated acid expressed in mmole acid/L aqueous

bread phase instead of mmole undissociated acid/ kg bread. No difference between antifungal activity of sourdough breads and chemically acidified bread with comparable $C_{HA,AA}$ concentrations was observed. Shelf-life significantly increased with 150 – 200 mmole undissociated AA/L. The role of $C_{HA,AA}$ in combination with the pH lowering effect of lactic acid is highly important. Future research can investigate the interaction effect of antifungal active sourdoughs in combination with other natural preservation strategies including efficient packaging and storage, *clean label* natural ingredients and ingredient optimization.

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Table 1 Sourdough characteristics: Dough Yield (DY), pH (n = 6), aw (n = 3), log CFU/g of yeasts and lactic acid bacteria (LAB) (n = 4), concentration of acetic acid (AA) and lactic acid (LA) (n = 3)

	A	B	C
DY	166.5	161	200
pH	4.9 ± 0.1[a]	4.0 ± 0.2[b]	4.1 ± 0.1[b]
a_w	0.98 ± 0.00[a]	0.96 ± 0.02[a]	0.98 ± 0.00[a]
Yeasts (log CFU/g)	7.1 ± 0.1[a]	5.3 ± 0.4[b]	7.1 ± 0.8[a]
LAB (log CFU/g)	8.7 ± 0.2[a]	9.0 ± 0.0[a]	9.0 ± 0.4[a]
conc AA (mmole/kg SD)	99 ± 2[a]	89 ± 1[a]	39 ± 1[b]
conc LA (mmole/kg SD)	53 ± 6[a]	85 ± 6[b]	53 ± 2[a]
FQ	0.5 ± 0.0[a]	1.0 ± 0.1[b]	1.4 ± 0.1[c]

[a-c] Values in the same row followed by different letters differ significantly ($p < 0.05$).

Table 2 Metagenomics analysis of lactic acid bacteria and yeasts/moulds in three industrial bio-sourdoughs

	A	B	C
Bacteria (%)			
Lactobacillus sanfranciscensis	**98.5**	**90.2**	2.6
Lactobacillales (order)	1	3.7	4.6
Lactobacillus sp.	0.4	2.1	0.9
Weissella confusa			**62.2**
Weissella salipiscis			1.8
Weissella sp.			9.8
Leuconostoc citreum			3.6
Other*	0.1	4	14.5
Sum	100	100	100
Yeasts/moulds (%)			
Saccharomyces cereviseae	0.3	**90.6**	**71.8**
Saccharomycetes (genus)	0.8	3.2	6.9
Kazachstania humilis (cfr. *Candida humilis*)	**96.8**		
Candida galli			5.1
Candida deformans			2.5
Eukaryota (kingdom)		4.9	2.4
Cladosporium ramotenellum		0.1	2
Kluyveromyces marxianus	0.5	0.5	1.1
Aureobasidium pullulans			1.7
Other*	1.6	0.7	6.5
Sum	100	100	100

* other: < 1 % of the sequences

Table 3 Mould free shelf-life (days) of par-baked (PB) and full-baked (FB) sourdough and chemically acidified wheat breads. Sourdough breads: 0 – 30 g sourdough/100 g dough; sourdough types A, B or C, or reference bread with 1500 mg propionic acid/100 g dough, packaged under air (n = 10).

		Sourdough bread	
	SD	**PB**	**FB**
	0	4.4 ± 0.6[a]	8.3 ± 4.0[a]
CaP	**0**	7.8 ± 1.4[bc]	24.4 ± 16.6[bcde]
A	**10**	7.6 ± 2.8[ab]	7.0 ± 0.0[ac]
	20	12.6 ± 3.8[b]	12.9 ± 4.8[ab]
	30	14.2 ± 4.8[b]	29.8 ± 16.6[abd]
B	**10**	4.0 ± 0.0[a]	15.6 ± 15.2[abd]
	20	9.0 ± 0.0[b]	31.2 ± 13.0[bd]
	30	13.7 ± 2.0[b]	49.0 ± 0.0[d]
C	**10**	5.7 ± 0.5[ac]	10.1 ± 3.5[ae]
	20	8.9 ± 2.1[b]	11.7 ± 4.4[ae]
	30	9.0 ± 1.6[b]	23.8 ± 18.0[abd]
		Chemically acidified bread	
series		**PB**	**FB**
	1	6.3 ± 1.0[a]	10.3 ± 4.2[a]
CaP	**2**	8.8 ± 1.4[ab]	49.0 ± 0.0[b]
	3	6.6 ± 1.3[a]	11.5 ± 7.0[a]
	4	8.7 ± 0.5[abc]	38.4 ± 11.4[b]
	5	6.9 ± 1.9[ad]	12.4 ± 3.1[a]
	6	9.6 ± 2.0[abc]	32.0 ± 15.9[ab]
	7	28.5 ± 16.5[bc]	49.0 ± 0.0[c]
	8	8.8 ± 2.3[ab]	30.3 ± 14.5[ab]
	9	7.3 ± 3.4[abc]	36.7 ± 13.5[b]
	10	49.0 ± 0.0[c]	49.0 ± 0.0[c]
	11	9.4 ± 3.9[ab]	37.1 ± 12.8[b]
	12	49.0 ± 0.0[c]	36.5 ± 11.8[b]
	13	49.0 ± 0.0[c]	49.0 ± 0.0[c]

[a-e] Values in the same column, grouped per category sourdough bread or chemically acidified bread, followed by different letters differ significantly ($p < 0.05$).

* Propionic acid (1500 mg PA/100 g dough) was added under the form of calcium propionate (CaP) due to its increased water solubility.

Table 4 Total concentrations (C_x) and undissociated acid concentrations ($C_{HA,x}$) of acetic (AA) and lactic (LA) acid in sourdough bread (sourdough type A, B or C (0 – 30 g sourdough/100 g dough) and pH (n = 8); par-baked (PB) or full-baked (FB) bread; determined in bread crust) (C_x expressed in mmole acid/kg bread crust and C_{HA} in mmole acid/L aqueous phase in bread crust) (n = 3).

		pH		C_{AA} (mmole/kg)		C_{LA} (mmole/kg)		$C_{HA,AA}$ (mmole/L)		$C_{HA,LA}$ (mmole/L)	
		PB	**FB**	**PB**	**FB**	**PB**	**FB**	**PB**	**FB**	**PB**	**FB**
	0	5.7 ± 0.2^{a}	5.8 ± 0.1^{a}	54 ± 4^{a}	60 ± 2^{a}	0 ± 0^{a}	0 ± 0^{a}	13 ± 1^{a}	15 ± 0^{a}	0 ± 0^{a}	0 ± 0^{a}
A	**10**	5.3 ± 0.1^{ab}	5.3 ± 0.1^{abc}	57 ± 4^{ab}	67 ± 1^{ab}	9 ± 1^{ac}	10 ± 3^{ac}	35 ± 2^{ab}	50 ± 1^{a}	1 ± 0^{ab}	1 ± 0^{a}
	20	5.0 ± 0.1^{b}	4.9 ± 0.3^{bcd}	65 ± 4^{bc}	73 ± 2^{bc}	18 ± 3^{ab}	19 ± 2^{ab}	38 ± 2^{ab}	108 ± 1^{a}	2 ± 0^{ab}	4 ± 0^{bc}
	30	5.0 ± 0.2^{b}	4.7 ± 0.2^{cd}	70 ± 5^{c}	80 ± 1^{c}	24 ± 2^{bd}	25 ± 3^{bd}	61 ± 4^{bd}	159 ± 1^{b}	3 ± 0^{ab}	3 ± 0^{ac}
B	**10**	5.2 ± 0.0^{ab}	5.0 ± 0.2^{bcd}	61 ± 4^{abc}	75 ± 7^{bc}	19 ± 1^{bc}	21 ± 4^{bc}	50 ± 4^{ab}	94 ± 9^{a}	3 ± 0^{bd}	3 ± 0^{ac}
	20	4.6 ± 0.2^{b}	4.6 ± 0.1^{cd}	71 ± 5^{c}	79 ± 1^{c}	32 ± 3^{bd}	34 ± 3^{bd}	121 ± 9^{cd}	172 ± 2^{b}	15 ± 1^{c}	19 ± 2^{d}
	30	4.3 ± 0.0^{b}	4.2 ± 0.1^{d}	75 ± 6^{c}	85 ± 2c	42 ± 4^{d}	49 ± 2^{d}	157 ± 14^{cd}	220 ± 5^{b}	30 ± 3^{c}	36 ± 3^{e}
C	**10**	5.2 ± 0.0^{ab}	4.9 ± 0.2^{bcd}	56 ± 4^{ab}	63 ± 3^{ab}	10 ± 1^{ac}	12 ± 5^{abc}	47 ± 3^{abd}	84 ± 3^{a}	1 ± 0^{ab}	2 ± 0^{ac}
	20	4.7 ± 0.2^{b}	4.7 ± 0.1^{cd}	58 ± 2^{ab}	66 ± 4^{ab}	19 ± 2^{abc}	20 ± 4^{abc}	91 ± 3^{bd}	139 ± 7^{b}	7 ± 1^{cd}	9 ± 1^{bc}
	30	4.7 ± 0.2^{b}	4.4 ± 0.3^{cd}	59 ± 2^{abc}	66 ± 4^{ab}	23 ± 3^{bd}	26 ± 6^{bd}	99 ± 4^{cd}	141 ± 9^{b}	9 ± 1^{cd}	9 ± 1^{bc}

$^{a-e}$ Values in the same column followed by different letters differ significantly ($p < 0.05$).

Table 5 Model parameters for the General Linear Regression Models of mould free shelf-life of sourdough breads and chemically acidified breads in function of SD type (A, B or C), baking condition (PB or FB), pH, a_w, and interaction terms. Equations y_1 and y_2 were developed for the data of the sourdough breads: y_1 with acid concentrations expressed in mmole/kg bread crust) (C_{TOT}) and y_2 with concentrations expressed in mmole undissociated acid/L aqueous phase in bread crust (C_{HA}). Eq. 3 (y_3) was developed using the shelf-life data of the chemically acidified breads; acid concentrations expressed in C_{HA}).

	Sourdough breads		**Chem. acidified breads**
Parameters	**y_1 (C_{TOT})**	**y_2 (C_{HA})**	**y_3 (C_{HA})**
Intercept	950	2473	-4.7
[SD = A]	8.5	6.0	/
[SD = B]	2.7	0.8	/
[SD = C]	0	0	/
[baking = PB]	-8.8		-3.6
[baking = FB]	0		0
pH	-159	-463	
a_w		-2607	
pH x a_w		488	
	x = C_{TOT}	**x = C_{HA}**	**x = C_{HA}**
$C_{x,AA}$	-13.9	0.1	0.2
$C_{x,LA}$	-6.1		
$C_{x,AA}$ x $C_{x,LA}$	0.1	0.0	
$C_{x,AA}$ x pH	2.3		
$C_{x,LA}$ x pH		-0.4	
R^2	**0.64**	**0.79**	**0.66**

* Parameter *packaging* (air versus MAP) was proven to be not significant ($p > 0.05$).

Table 6 Concentrations of total acid (acetic and lactic acid) added in chemically acidified wheat bread (C_{TOT}: mmole/kg bread) or reference bread with 1500 mg propionic acid/100 g dough; and concentrations of undissociated (C_{HA}) acetic and lactic acid in par-baked (PB) or full-baked (FB) wheat bread, calculated in the bread crust*) (mmole acid/L aqueous phase in bread crust). The pH of the breads is also shown as this was a very important parameter for calculating the concentration of undissociated acid (n = 8)

	pH		$C_{TOT,AA}$	$C_{TOT,LA}$	$C_{HA,AA}$ (mmole/L)		$C_{HA,LA}$ (mmole/L)	
series	**PB**	**FB**	**(mmole/kg)**	**(mmole/kg)**	**PB**	**FB**	**PB**	**FB**
1	5.4 ± 0.1^{ab}	5.6 ± 0.1^{a}	57	0	31	24	0	0
2 (CaP)	5.6 ± 0.1^{a}	5.6 ± 0.1^{a}	57	0	21	24	0	0
3	4.9 ± 0.1^{abc}	4.9 ± 0.1^{ab}	75	0	91	107	0	0
4	4.5 ± 0.0^{abcd}	4.4 ± 0.1^{abc}	100	0	188	238	0	0
5	4.7 ± 0.0^{abcd}	4.7 ± 0.1^{abc}	57	25	89	104	9	11
6	4.3 ± 0.1^{cd}	4.3 ± 0.1^{abcd}	75	25	163	191	20	23
7	4.1 ± 0.1^{cd}	4.1 ± 0.1^{bcd}	100	25	240	282	27	31
8	4.4 ± 0.1^{bcd}	4.2 ± 0.0^{bcd}	57	50	116	153	33	54
9	4.2 ± 0.2^{cd}	4.2 ± 0.1^{bcd}	75	50	172	202	46	54
10	4.1 ± 0.2^{cd}	4.0 ± 0.2^{bcd}	100	50	240	293	54	72
11	4.0 ± 0.1^{cd}	4.0 ± 0.2^{bcd}	57	75	142	167	93	109
12	4.0 ± 0.1^{d}	3.9 ± 0.1^{cd}	75	75	187	227	93	123
13	3.8 ± 0.1^{d}	3.7 ± 0.1^{cd}	100	75	264	317	118	153

[a-d] Values in the same column followed by different letters differ significantly ($p < 0.05$); *No standard deviations of the total concentrations of acids (C_{tot})can be given because these values are mathematically derived from the equations determined between the concentration of acid in dough and in bread. Therefore there are also no standard deviations of the concentrations of undissociated acid (C_{HA}).*

** Moisture content in bread crust of par-baked and full-baked breads were respectively 34 ± 3 and 29 ± 3 % (n = 20).*

** Propionic acid (1500 mg PA/100 g dough) was added under the form of calcium propionate (CaP) due to its increased water solubility

Table 7 Bread quality characteristics of sourdough bread (sourdough type A, B or C (0 – 30 g sourdough/100 g dough) and chemically acidified wheat bread; par-baked (PB) or full-baked (FB) bread or reference bread with 1500 mg propionic acid/100 g dough: bread weight (g) and volume (mL) (n = 10 - 20).

		sourdough bread			
		weight		volume	
	SD	PB	FB	PB	FB
	0	61 ± 10^{a}	62 ± 9^{a}	159 ± 21^{ab}	160 ± 18^{ab}
CaP	0	64 ± 10^{a}	63 ± 10^{a}	141 ± 20^{a}	142 ± 16^{a}
A	10	62 ± 9^{a}	56 ± 11^{b}	184 ± 15^{bc}	167 ± 17^{a}
	20	67 ± 12^{a}	59 ± 13^{ab}	209 ± 34^{cd}	184 ± 30^{bc}
	30	63 ± 11^{a}	55 ± 9^{b}	196 ± 25^{cd}	176 ± 19^{bc}
B	10	69 ± 4^{a}	61 ± 8^{ab}	189 ± 8^{bcd}	174 ± 18^{bc}
	20	67 ± 4^{a}	66 ± 6^{a}	196 ± 7^{cd}	182 ± 12^{abc}
	30	72 ± 5^{a}	64 ± 9^{a}	170 ± 18^{bc}	154 ± 22^{ab}
C	10	60 ± 12^{a}	57 ± 8^{b}	202 ± 33^{cd}	193 ± 16^{c}
	20	63 ± 8^{a}	57 ± 7^{b}	234 ± 20^{d}	204 ± 24^{c}
	30	61 ± 7^{a}	57 ± 9^{b}	216 ± 22^{d}	197 ± 29^{c}
		chemically acidified bread			
		weight		volume	
	series	PB	FB	PB	FB
	1	61 ± 10^{ad}	53 ± 10^{a}	190 ± 24^{a}	168 ± 19^{a}
CaP	2	79 ± 2^{bc}	63 ± 9^{a}	191 ± 4^{a}	146 ± 23^{ab}
	3	68 ± 9^{abd}	70 ± 1^{ab}	187 ± 25^{ab}	183 ± 2^{ab}
	4	67 ± 6^{abcd}	66 ± 4^{b}	103 ± 9^{bc}	106 ± 13^{bcd}
	5	60 ± 3^{abcd}	58 ± 8^{a}	194 ± 6^{ab}	151 ± 25^{abc}
	6	72 ± 10^{c}	66 ± 2^{a}	140 ± 2^{abc}	130 ± 4^{bcd}
	7	58 ± 10^{d}	71 ± 1^{b}	77 ± 12^{c}	97 ± 5^{cd}
	8	69 ± 11^{d}	64 ± 6^{b}	151 ± 13^{abc}	139 ± 17^{abc}
	9	62 ± 10^{abcd}	63 ± 3^{ab}	105 ± 13^{bc}	98 ± 4^{cd}
	10	71 ± 5^{abcd}	64 ± 1^{a}	72 ± 5^{c}	79 ± 2^{d}
	11	56 ± 2^{d}	58 ± 6^{a}	102 ± 4^{bc}	107 ± 13^{bcd}
	12	69 ± 4^{abc}	63 ± 0^{ab}	95 ± 1^{c}	96 ± 1^{d}
	13	69 ± 5^{abc}	59 ± 3^{a}	82 ± 7^{c}	77 ± 2^{d}

[a-d] Values in the same column, grouped per category sourdough bread or chemically acidified bread, followed by different letters differ significantly ($p < 0.05$)

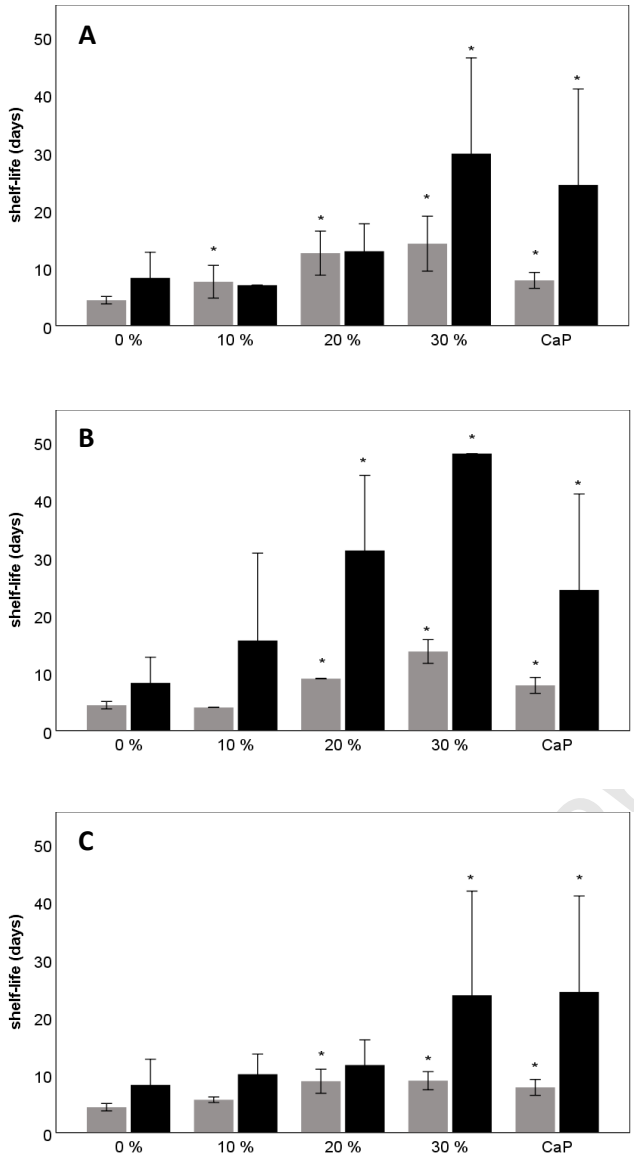

Figure 1 Mould free shelf-life (days) of par-baked (PB, grey) and full-baked (FB, black) breads with and without sourdough (0 – 30 g sourdough/100 g dough (%); sourdough types A, B or C) or reference bread with 1500 mg propionic acid/100 g dough. Propionic acid added under the form of calcium propionate (CaP), packaged under air atmosphere(n = 10) (*: indication of significant difference of shelf-life compared to the control (0 %)).

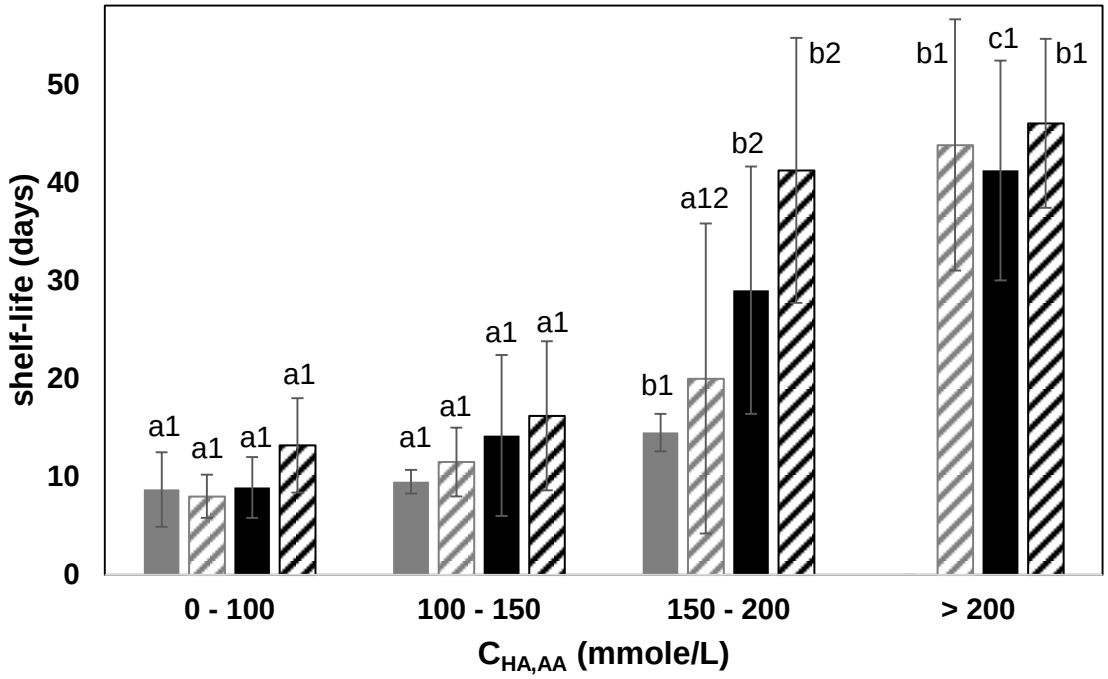

Figure 2 Mould free shelf-life (days) of par-baked (PB, grey) and full-baked (FB, black) sourdough breads (plain) and chemically acidified breads (arced), expressed in terms of $C_{HA,AA}$ (0 - 200 mmole/L aqueous phase in bread crust). Values produced with the same baking conditions (PB/FB and SD bread/chem. acid. bread) differ significantly ($p < 0.05$) if they do not share a common superscript letter. Values within similar ranges of $C_{HA,AA}$ differ significantly when they do not share a common superscript number. There was no data available for PB sourdough bread with $C_{HA,AA}$ > 200 mmole/L.

Highlights:

- The role of undissociated acetic and lactic acid in bread preservation was shown.
- Antifungal concentrations of organic acids must be expressed on the aqueous phase.
- Bread shelf-life increased when > 150 mmole undissociated acetic acid was present.

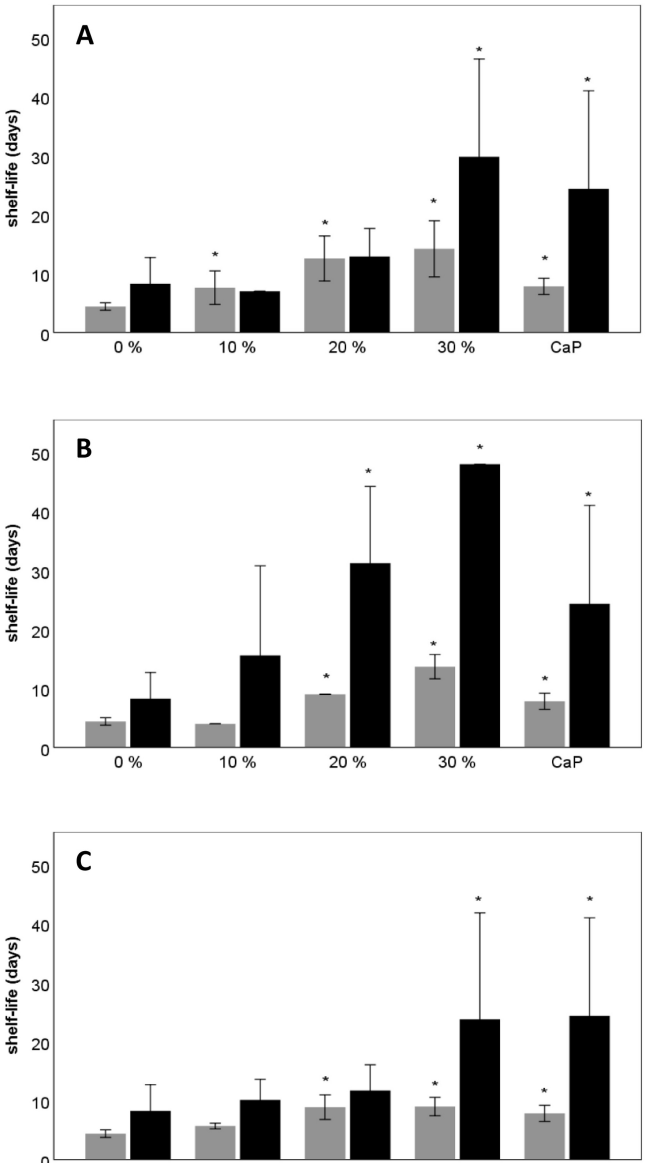

Figure 1

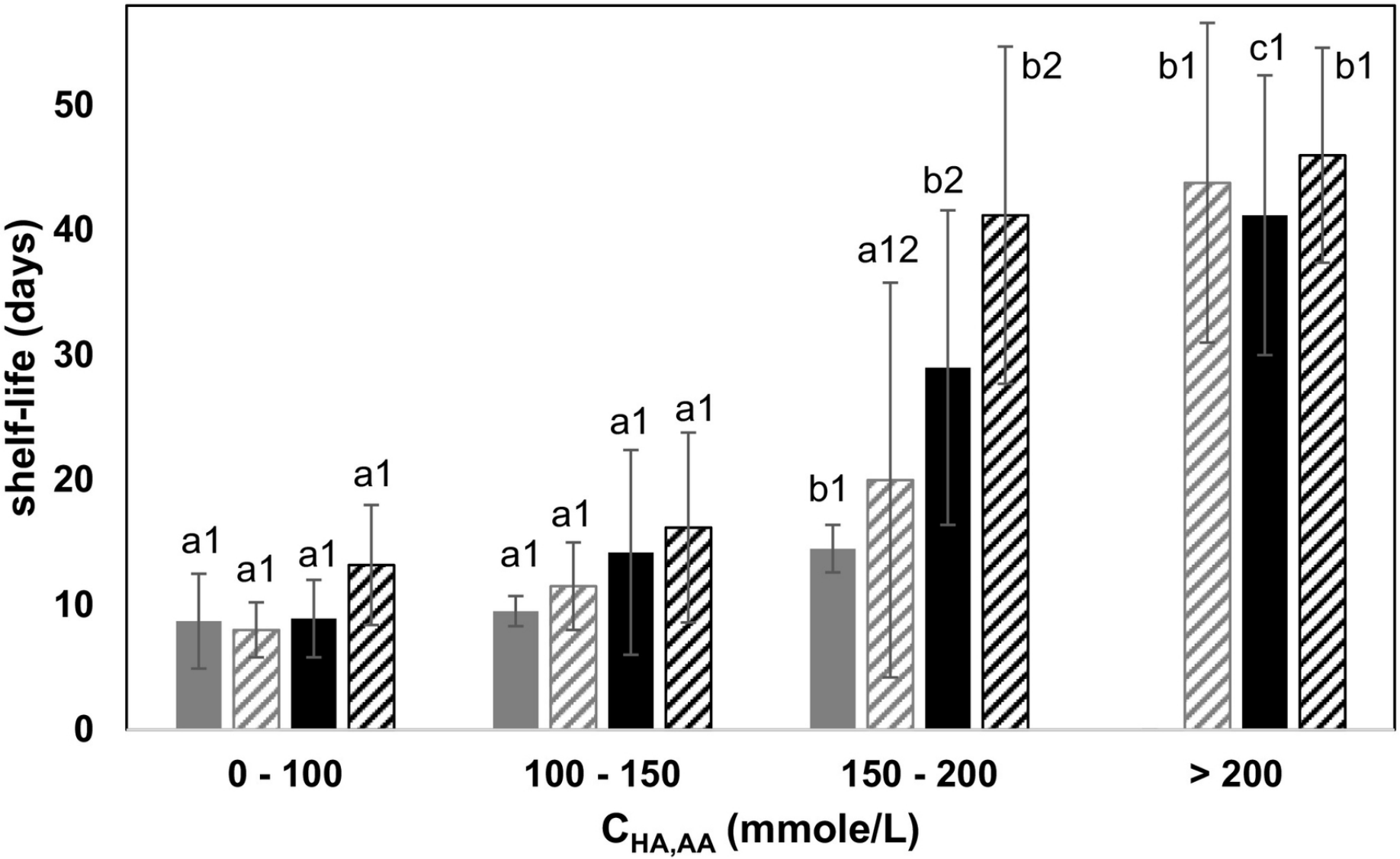
shelf-life (days)
0
10
20
30
40
50
a1
a1
a1
a1
a1
a1
a1
b1
a12
b2
b2
b1
c1
b1
0 - 100
100 - 150
150 - 200
> 200
$C_{HA,AA}$ (mmole/L)

Figure 2