Impact of par-baking and packaging on the microbial quality of par-baked wheat and sourdough bread

Els Debonne a, b, *, Filip Van Bockstaele a, Manon Van Driessche a, b, Ingrid De Leyn a, Mia Eeckhout a, b, Frank Devlieghere b, c

a Research Unit of Cereal and Feed Technology, Department of Food Technology, Safety and Health, Faculty of Bioscience Engineering, Ghent University, Valentin Vaerwyckweg 1, 9000 Ghent, Belgium
b Laboratory of Applied Mycology (MYCOLAB), Department of Food Technology, Safety and Health, Faculty of Bioscience Engineering, Ghent University, Valentin Vaerwyckweg 1, 9000 Ghent, Belgium
c Laboratory of Food Microbiology and Food Preservation, Department of Food Technology, Safety and Health, Faculty of Bioscience Engineering, Ghent University, Coupure Links 653, 9000 Ghent, Belgium

* Corresponding author. Research Unit of Cereal and Feed Technology, Department of Food Technology, Safety and Health, Faculty of Bioscience Engineering, Ghent University, Valentin Vaerwyckweg 1, 9000 Ghent, Belgium. E-mail address: els.debonne@ugent.be (E. Debonne).

ARTICLE INFO

Article history:
Received 17 January 2018
Received in revised form 21 March 2018
Accepted 22 March 2018
Available online 23 March 2018

Keywords:
Microbiological quality
Par-baking
Bread
Moulds and yeasts
Modified atmosphere packaging
Shelf life
Par-baked bread

Abstract

The impact of processing conditions on the microbial quality of par-baked wheat and sourdough bread was investigated. Processing conditions included par-baking time (8 and 13 min), temperature (150 and 200 °C), amount of steam (200 and 600 mL), and packaging (air and modified atmosphere (MA)). Total anaerobic mesophilic plate counts, moulds and yeasts and spore-forming bacteria, together with pH and aw of the par-baked breads were evaluated. Data were used to make predictive models showing the impact of the main effects and their interactions. Sourdough addition could extend the time of acceptable bread quality based on the anaerobic counts from 8 to more than 13 days. Visual growth of moulds and yeasts (presence/absence of single spots) was most efficiently suppressed by the combination of MA-packaging and the highest baking temperature and time. Microbiological analysis of moulds and yeasts however, showed that again sourdough had the best preservation potential, followed by MA-packaging. This study showed that adjusting the par-baking conditions, bread composition and packaging can increase the shelf-life of par-baked bread in a natural way.

© 2018 Elsevier Ltd. All rights reserved.

1. Introduction

Par-baking is a widely used strategy for bakery products, as it enables to achieve longer food shelf-lives, both technological as microbiological (Almeida, Steel, & Chang, 2016). The two-phase baking procedure either results in (1) a softly baked pale product after the first par-baking phase which is packaged and can be stored at room, cooled or frozen temperatures, and (2) in a brown, crispy and freshly perceived product after the second fully baking phase (Debonne, Van Bockstaele, Philips, De Leyn, & Eeckhout, 2017). Par-baked breads intended to be stored at room temperature are mostly MA-packaged (modified atmosphere), with CO2, N2 and without oxygen. The par-baking strategy can decrease the amount of bread waste by providing fresh bread at any wanted moment of the day. The main reason for bread rejection is staling, which is generally perceived as a crumb hardness increase (Bárcenas & Rosell, 2006; Eckardt et al., 2013). However, par-baked breads are mostly rejected because of visible mould or yeast spoilage on the outer layer of the breads (Deschuyfleer et al., 2011; Lainez, Vergara, & Bárcenas, 2008). Mould growth is by far the most important microbiological shelf-life limiting factor of bread products, with Penicillium spp., Aspergillus spp. and Fusarium spp. being the most dominant species (Gerez, Torino, Rollán, & Font de Valdez, 2009; Legan, 1993). Spoilage of bread can also be caused by chalk yeasts (cf. chalk moulds) which are spoilage yeasts that cause chalk mould defects (dust-type spots) on bread (Deschuyfleer et al., 2011). Next to mould and yeast spoilage, the formation of rope as a result of growth of the spore forming bacterium Bacillus subtilis, usually present in raw bakery materials, can also result in the rejection of bread products. Spores of Bacillus can survive the baking process, after which they can potentially cause spoilage of the baked bread.
product (Devlieghere, Debevere, Jacksens, Uyttendaele, & Vermeulen, 2011). Smith, Daifas, El-Khouri, Koukoutsis, and El-Khoury (2004) provided a thorough review on microbial control measures for bakery products, with the main focus on technological strategies to reduce post-baking contamination and the use of chemical preservatives. The most used chemical preservatives for bread are propionic acid and its salts (Pattison, Lindsay, & Von Holy, 2004). Despite the fact that the spectrum of activity of sorbic acid with regard to spoilage yeasts and moulds is wider than that of propionic acid (Devlieghere et al., 2011), the use of sorbic acid is limited as it affects the activity of yeasts in leavened dough. Next to the use of chemical preservatives, the use of sourdough is also a widely used strategy for the extension of bread shelf-life. The main activity is hypothesized to result from the formation of organic acids such as lactic and acetic acid (Axel et al., 2016; Le Lay, Mounier, et al., 2016). Another explanation for the antifungal activity of sourdough can be found within the activity of the yeasts in the sourdough since different yeasts such as Wickerhamomyces anomalous and Meyerozyma guilliermondii have already been reported to exert antifungal activity in bread. The antifungal effect has been attributed to the formation of ethyl acetate and ethanol during sourdough production (Coda et al., 2011, 2013).

The microbiological shelf-life of par-baked breads has previously been reported in function of the par-baking time (Karaoglu, Kotancilar, & Gurses, 2005), storage time and temperature (Karaoglu et al., 2005; Lainez et al., 2008), rebaking conditions (time and temperature) (Leuschner, O’Callaghan, & Arendt, 1999) and sourdough (Gerez et al., 2009; Torrieri, Pepe, Ventorino, Masi, & Cavella, 2014). However, most research mainly studied the impact of the baking conditions, storage and composition on the technological quality of the par-baked breads instead of microbiological quality (Debonne et al., 2017; Karaoglu, 2006; Majzooobi, Farahmaky, & Agah, 2011; Vulicevic, Abdel-Aal, Mittal, & Lu, 2004). Additionally, the combined effect of sourdough, par-baking conditions and packaging on the microbiological shelf-life has not been described before, although being emphasized in Guynot, Marin, Sanchis, and Ramos (2003) as very important due to potential synergistic or additive effects.

In this article, the authors aimed to acquire further knowledge regarding the impact of par-baking conditions, sourdough, packaging and storage on the microbiological quality of par-baked bread. The results of this research are complementary to the results in Debonne et al. (2017), where the impact of par-baking and storage conditions on the technological quality of par-baked and fully baked wheat bread was investigated.

2. Materials and methods

2.1. Experimental set-up

In this study wheat breads, with or without sourdough, were produced and par-baked under varying par-baking conditions, including baking time, baking temperature and steam. Further, they were packaged under either air or modified atmosphere. To determine the impact of each parameter on bread microbiological quality, two extreme values of each parameter were chosen based on Debonne et al. (2017) (par-baking conditions) and Debonne, Van Bockstaele, De Leyn, Devlieghere, and Eckhout (2018) (sourdough) (time: 8, 13 min; temperature: 150, 200 °C; steam: 200, 600 ml; sourdough: 0, 30 g SD/100 g bread dough). The combination of all these parameters resulted in eight different baking programs and 32 different subgroups (8 baking programs x 2*SD) x 2*packaging. Per baking test, 30 small breads (70 g dough) were produced. After baking, the group was divided into two, resulting in 15 replicates per baking test and per packaging condition. All packaged breads were stored at 22 °C to simulate storage at room temperature. During storage, samples of each of the 16 different baking tests were taken on days 3, 8 and 13 after baking. The microbiological analysis consisted of determining the total mesophilic anaerobic plate count (TAPC), moulds and yeasts count (M & Y) and spore forming bacteria counts. All experiments were performed twice. Parallel, breads were daily checked for visual mould or yeast spoilage (shelf-life test). The data of all quality parameters were modelled in order to assess the impact of all conditions combined.

2.2. Bread making procedure

All experiments were performed 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. Sourdough was provided by L’Atelier du Pain (Ninove, Belgium). The sourdough was a type 1 sponge dough (De Vuyst et al., 2014). 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, 60.3 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), 0.1 g malt flour and 5 mg ascorbic acid/100 g flour. In case sourdough was added (30 g SD/100 g dough), the amounts of flour and water added through the sourdough were adjusted to result in an optimal water/flour mixing ratio. Ingredients were mixed in a De Danielli 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 80–90% relative humidity (RH). Dough was divided (30 ± 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 90 min at 30 °C and 80–90% 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). Baking conditions were varied and are listed in Table 1. The ventilation of the oven was set at the highest ventilation speed. The baking temperature of the second phase of par-baking was set 20 °C lower than the first phase. For example, baking temperatures of phase 1 and 2 of a baking condition were respectively 170 and 150 °C. Par-baking time of the second phase and steam volume were varied as well. Total par-baking time was 10 or 15 min. Throughout this study, baking time and temperature always refers to the 2nd baking phase of par-baking.

The breads were then cooled to room temperature in the bakery environment. This led to a natural post-contamination of the breads with airborne moulds and yeasts. Furthermore, the breads were stored at room temperature (22 °C) in sealed plastic bags (PA/PE/20/70) (PA: polyamide; PE: polyethylene) or packaged under modified atmosphere (MA) with a Tray Sealer (DECA Packaging Group, Herentals, Belgium) using a gas composition of 70% CO₂ and 30% N₂. The breads were MA-packaged per two in a tray (632 mL) made out of PP/EVOH/PP (PP: polypropylene; EVOH: ethylene vinylalcohol) and sealed with a cover film of OPA/EVOH/PE/OPA (OPA: orientated polyamide).

<table>
<thead>
<tr>
<th>Phase</th>
<th>Time (min)</th>
<th>Temperature (°C)</th>
<th>Steam volume (mL)</th>
<th>Steam valve</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>170/220</td>
<td>200/600</td>
<td>Closed</td>
</tr>
<tr>
<td>2</td>
<td>8/13</td>
<td>150/200</td>
<td>0</td>
<td>Open</td>
</tr>
</tbody>
</table>

Table 1 Par-baking process parameters.
2.3. Bread evaluation

2.3.1. pH and aw

The impact of the different bread production and par-baking conditions on pH and aw was evaluated by measuring the pH with a portable pH meter (model HI 83141, Hanna Instruments) and aw with a LabMaster-Aw (Novasina). Bread samples for aw analysis were taken from the crumb as in Debonne et al. (2018). No significant difference was found between crumb and crust aw of par-baked bread.

2.3.2. Microbiological analyses

2.3.2.1. Sourdough characterization. The composition of the sourdough, which consists of lactic acid bacteria and yeasts, was determined by an external company (Genalyse Partner) through the use of metagenomics. This metagenomics analysis was achieved using a Next Generation Sequencer (illumina Miseq). Genalyse Partner performed a direct DNA extraction from the sourdough. 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 Liege University. Moreover, on day 0, the microbial load (log cfu/g) of the ripe sourdough was determined.

2.3.2.2. Bread and sourdough quality. On days 3, 8 and 13 after baking the microbial quality of the par-baked breads was assessed. 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. For the sourdough analysis, 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 lactic acid bacteria and yeasts respectively. The breads were analyzed on Plate Count Agar (PCA, Oxoid CM0325) for the anaerobic count and spore forming bacteria and OGY for moulds and yeasts. For spore forming bacteria detection, the dilutions were subjected to a thermal heat treatment of 10 min at 80 °C in a hot water bath (Butcher et al., 2015). PCA plates for total anaerobic plate count and MRS plates for the lactic acid bacteria in the sourdough 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. Plates for spore counts were incubated for 3 days at 30 °C as well. OGY pates were incubated for 3 days at 25 °C (Gül, Öçelik, Sağdıç & Certel, 2005). Dilution series of the bread samples were made (dilution –1 to –4). Plates were considered countable when the number of colony forming units ranged between 20 and 300 cfu/plate. The detection limits (lower and upper) of the pour-plates for the bread quality analyses were: LL = 2.3 log cfu/g (20 cfu/plate at dilution –1) and UL = 6.5 log cfu/g (300 cfu/plate at dilution –4). For the determination of the microbial load of the sourdough, a dilution series of –4 to –7 was prepared.

2.4. Statistical analysis

To assess significant differences among samples, a multiple comparison analysis of samples was performed using SPSS Statistics version 23. In case the results were normally distributed, either a Tukey test (homoscedasticity) or Dunnett T3 test was used to describe the means with 95% confidence (p = 0.05). A Dunn test for multiple comparisons was applied, preceded by a non-parametric Kruskal-Wallis 1-one ANOVA, for non-normally distributed data. Assuming there is a linear relationship among the main effects of baking parameters (e.g. x1), storage condition (e.g. x2), the interaction effects (e.g. x1*x2) and the quality parameters (e.g. y1) assessed for bread quality, the GLM (General Linear Model) Univariate procedure was performed on the data (e.g. equation GLM: y1 = intercept + a∗x1 + b∗x2 + c∗x1∗x2) (Debonne et al., 2017).

3. Results and discussion

3.1. Sourdough characteristics

The ripe sourdough showed following characteristics: dough yield (DY) of 166.5, pH 4.49 ± 0.25 and aw 0.967 ± 0.009 (n = 6). Moreover, the sourdough contained 9.2 ± 0.2 log lactic acid bacteria and 6.0 ± 0.4 log yeasts (n = 12). One dominant lactic acid bacteria isolate was obtained from the sourdough. The lactic acid bacteria were identified as Lactobacillus sanfranciscensis (98.5%). The yeasts present in the sourdough were identified as Kazachstania humilis (syn. Candida humilis) (96.8%), Saccharomyces (genus) (0.8%), K. exigua (0.7%), Kluyveromyces marxianus (0.5%), Saccharomyces barnetti (0.4%), S. cerevisiae (0.3%), K. turicensis (0.2%) and Candida sp. (0.1%). K. humilis can metabolize many sugars but is unable to break down maltose, which is the predominant sugar in sour- doughs. However, L. sanfranciscensis can break down the maltose into fructose and glucose that will be used by K. humilis (Van Kerrebroeck, Maes, & De Vuyst, 2017).

3.2. Effect of par-baking conditions and sourdough on pH and aw of par-baked bread

The pH of the breads was significantly reduced by the sourdough, from pH 5.81 for the wheat breads to 4.89 for the sourdough breads (p < 0.001) (Table 2). The pH drop of the sourdough bread is significantly reduced by the sourdough, which consists of lactic acid bacteria and yeasts, was determined by an external company (Genalyse Partner) through the use of metagenomics. This metagenomics analysis was achieved using a Next Generation Sequencer (illumina Miseq). Genalyse Partner performed a direct DNA extraction from the sourdough. 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 Liege University. Moreover, on day 0, the microbial load (log cfu/g) of the ripe sourdough was determined.

Table 2: Influence of par-baking conditions (baking temperature and time) on the pH and aw of the par-baked breads.

<table>
<thead>
<tr>
<th>Sourdough</th>
<th>T (min)</th>
<th>Time (h)</th>
<th>pH (±0.1)</th>
<th>aw (±0.005)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wheat</td>
<td>150</td>
<td>8</td>
<td>5.81 ± 0.10</td>
<td>0.969 ± 0.005</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>8</td>
<td>5.81 ± 0.10</td>
<td>0.958 ± 0.005</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>8</td>
<td>5.81 ± 0.10</td>
<td>0.952 ± 0.005</td>
</tr>
</tbody>
</table>

*Values in the same column followed by different letters differ significantly (p < 0.05).
3.3. Microbiological analyses of par-baked bread

3.3.1. Total mesophilic anaerobic bacteria

Total anaerobic plate counts (TAPC) were determined as par-baked breads are mostly packaged under modified atmosphere with limited oxygen (Khoshakhlagh, Hamdami, Shahedi, & Le-Bail, 2014). The results were expressed in colony forming units (cfu) per gram of bread. In Fig. 1 (600 mL steam), it can be seen that the sourdough had a large impact on the TAPC with almost complete growth inhibition during storage time of 13 days. In case of bacteria, this is largely due to the pH lowering effect of the sourdough (Messens & De Vuyst, 2002; Voysey & Hammond, 1993, pp. 80-94).

In Belgium, quality limits for bread are set by the Federal Agency for Safety of the Food Chain (FAVV, Belgium). The limits are expressed as m and M, respectively m being the limit under which bread quality is guaranteed and M being the maximally allowed upper limit. For the TAPC, m is 4 and M 5 log cfu/g bread. In Fig. 1 it is shown that based on the TAPC, bread quality after 13 days is acceptable when sourdough is used, or without sourdough but baked at 200 °C for 8 or 13 min. A higher baking temperature, reduces the amount of bacteria resulting in a better bread quality. The TAPC was almost not influenced by the packaging strategy. Besides, it is important to note that the breads in this study are par-baked and the limits are for fully baked breads. The flora present will be reduced upon the final baking stage of par-baked breads. Additionally, fully baked bread will have a lower aw (Karaoğlu et al., 2005) which will be an extra hurdle for the bacteria (Leistner & Gorris, 1995).

3.3.2. Moulds and yeasts

Visual bread spoilage by moulds and yeasts is the major reason of rejection of bread by the consumer (Legan, 1993). Therefore, next to the microbiological analysis of moulds and yeasts, the visual bread shelf-life was determined. From the moment a single spot of mould or yeast was detected on the bread, it was considered spoiled (Table 4). After 4 days, most air packaged breads were spoiled. This is not in accordance with previously published results in Debonne et al. (2018), however, the mould free shelf-life corresponds to challenged breads (inoculated with two log spores of moulds Penicillium paneum or Aspergillus niger), indicating an increase of natural contamination in the bakery environment. Only the combination of baking temperature (200 °C) and time (13 min), resulted in a shelf-life increase of 2-4 days. Due to the higher dough weights (70 g) compared to the dough weights used in Debonne et al. (2018) (65 g), less head space was available resulting in a less effective modified atmosphere for the control of post-

Table 3
GLM of the microbiological quality parameters of par-baked bread (yi; pH and aw) in function of par-baking conditions xi; baking temperature T (°C), time (min) and steam (S, mL) conditions used during the 2nd baking-phase of par-baking; sourdough (g/100 g bread dough).

<table>
<thead>
<tr>
<th></th>
<th>pH</th>
<th>aw</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercep</td>
<td>5.806</td>
<td>1.018</td>
</tr>
<tr>
<td>T</td>
<td>2.40E-4</td>
<td>0.002</td>
</tr>
<tr>
<td>time</td>
<td>-0.030</td>
<td>1.46E-4</td>
</tr>
<tr>
<td>sourdough</td>
<td>0.96</td>
<td>0.65</td>
</tr>
<tr>
<td>R²</td>
<td>0.96</td>
<td>0.65</td>
</tr>
</tbody>
</table>

Fig. 1. Microbiological analysis of total mesophilic anaerobic plate counts (TAPC) of par-baked breads with or without 30 g sourdough/100 g bread dough and packaged under air or MAP atmosphere, & with a fixed amount of steam (600 mL). Four combinations of par-baking and temperature (i) 8 min—150 °C (black bars), (ii) 13 min—150 °C (grey bars), (iii) 8 min—200 °C (horizontally striped bars), and (iv) 13 min—200 °C (vertically striped bars).
baking moulds and yeasts contamination (Fernandez, Vodovotz, Courtney, & Pascall, 2006). The shelf-life test showed no significant effect of sourdough ($p > 0.05$). On the other hand, microbiological analysis of moulds and yeasts showed a clear effect of the sourdough (Fig. 2). This contradiction is due to the fact that the shelf-life test rejected breads from the moment a single spot was detected, regardless of the number of spots. Sourdough resulted in a longer lag phase time (Fig. 2B, D), hereby extending the time of acceptable quality ($m = 3$ and $M = 4$ log cfu/g (FAVV, Belgium)). In case of moulds and yeasts, the effect of sourdough is little or not pH dependent (Debonne et al., 2018; Sautour, Rouget, Dantigny, Divies, & Bensoussan, 2001). Several reasons for antifungal activity of sourdough have been reported, such as (1) formation of organic acids (Le Lay, Coton, et al., 2016), (2) phenyllactic acid (Valerio, Di Biase, Lattanzio, & Lavermicocca, 2016) and (3) hydroxyl fatty acids (Black, Zannini, Curtis, & Gänzle, 2013).

### 3.3. Spore forming bacteria

In Fig. 3, counts of spore forming bacteria, among which *Bacillus subtilis* is the most known in bread products, are represented. Bacteria of *B. subtilis* are gram-positive rod-shaped microorganisms which can form spores (Tan & Ramamurthi, 2014). Moreover, these spores are heat resistant and can therefore survive the heating process of baking (Setlow, 2006). The use of sourdough resulted in almost no detection of spore forming bacteria, with 3 log being the highest recorded value. Only in the case of breads baked without sourdough, 200 mL steam for 8 min at 150°C and air-packaged (Fig. 3A), growth of spore forming bacteria was observed during storage. Other results showed that next to the use of sourdough, MA-packaging and baking time can help suppress growth. A baking time/temperature combination of 2 min 170°C + 13 min 150°C (total baking time = 15 min) was sufficient to reduce almost all counts of spore forming bacteria. MA-packaging showed similar effect on controlling these bacteria, therefore both parameters contributed equally to the inhibition of spore formers. Bailey and Von Holy (1993) investigated storage

### Table 4

<table>
<thead>
<tr>
<th>Pack</th>
<th>sourdough</th>
<th>T</th>
<th>Time</th>
<th>Shelf-life</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>150</td>
<td>8</td>
<td>4.0 ± 0.0$^a$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13</td>
<td>4.0 ± 0.0$^a$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>200</td>
<td>8</td>
<td>4.7 ± 0.5$^b$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13</td>
<td>4.7 ± 0.5$^b$</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
<td>150</td>
<td>8</td>
<td>4.0 ± 0.0$^a$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13</td>
<td>4.0 ± 0.0$^a$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>200</td>
<td>8</td>
<td>4.8 ± 0.8$^b$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13</td>
<td>4.8 ± 0.8$^b$</td>
<td></td>
</tr>
<tr>
<td>MAP</td>
<td>0</td>
<td>150</td>
<td>8</td>
<td>6.0 ± 0.8$^b$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13</td>
<td>5.6 ± 0.8$^b$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>200</td>
<td>8</td>
<td>7.1 ± 1.2$^b$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13</td>
<td>11.1 ± 6.0$^{a,b}$</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
<td>150</td>
<td>8</td>
<td>6.3 ± 0.6$^b$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13</td>
<td>6.5 ± 0.9$^b$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>200</td>
<td>8</td>
<td>10.5 ± 6.3$^{a,b}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13</td>
<td>13.8 ± 6.0$^{a,b}$</td>
<td></td>
</tr>
</tbody>
</table>

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

Fig. 2. Microbiological analysis of moulds and yeasts of par-baked breads with or without 30 g sourdough/100 g bread dough and packaged under air or MAP atmosphere, & with a fixed amount of steam (600 mL). Four combinations of par-baking and temperature (i) 8 min–150°C (black bars), (ii) 13 min–150°C (grey bars), (iii) 8 min–200°C (horizontally striped bars), and (iv) 13 min–200°C (vertically striped bars).
temperature and found that storage at 30°C leads to a strong increase of Bacillus counts. In this study, all breads were stored at 22°C. It is possible that when the storage temperature is increased, this will result in differences in effects of the baking parameters, packaging and sourdough. Moreover, it had been researched that the 2nd baking phase of par-baked breads can result in 1 log reduction (Bailey & Von Holy, 1993).

3.4. General Linear Models (GLM)

The TAPC, M & Y and spores of B. subtilis data were used in predictive models to describe the effect of par-baking conditions, sourdough and packaging on the cell counts (Table 5). Both single and interaction effects among the parameters were included in the models. Model fitness $R^2$ shows the extent to which the models correlate with the experimental data. The combination of all five parameters (baking time, temperature, steam, sourdough and packaging) resulted in model fitness values ranging from 0.82 to 0.93 (TAPC), 0.77 to 0.89 (M & Y) and 0.51 to 0.94 (spore forming bacteria) for the models of days 3, 8 and 13. It can be concluded that all the tested parameters can predict the microbiological quality parameters correctly. Results also highlight that microbiological quality of par-baked bread results from a complex interaction between different factors and that it is difficult to select only one parameter as preservation strategy. Other factors, outside the scope of this article, affecting the microbiological quality are related to storage temperature, relative humidity, ingredient quality, good hygienic working practices in the laboratories and sampling variability.

4. Conclusion

The influence of par-baking conditions (baking time, temperature and steam) and packaging on the microbiological quality of par-baked wheat and sourdough bread was investigated. These parameters were able to predict the microbiological quality of the par-baked breads to a high extent. Model $R^2$ ranged between 0.51 and 0.94. The number of anaerobic bacteria was greatly reduced by the addition of sourdough. Sourdough addition resulted in a pH decrease of the par-baked breads (from pH 5.81 to 4.89). Based on the TAPC results, par-baked bread quality was acceptable for the sourdough breads or for the breads baked at 200°C (total baking time was 2 (1st baking phase) + 8 (2nd baking phase) = 10 min). Through the shelf-life test, where visual mould/yeast spoilage set the limit of quality, the impact of the different parameters was less pronounced compared to the microbiological analysis of moulds and yeasts. In the former method, the combination of MAP-packaging and the highest baking temperature and time ($\Delta T_{max}$) showed the best preservation potential. In the latter method, the microbiological analysis, sourdough had a lag phase extending effect, indicating active mechanisms working upon mould and yeast growth. This needs to be explored further in order to clearly elucidate upon the activity of this particular sourdough type. Generally, it can be concluded that for the different microbiological quality parameters the following two parameters had the biggest impact: (1) mesophilic anaerobic bacteria: sourdough and baking temperature, (2) moulds and yeasts: sourdough and packaging and (3) spore forming bacteria: sourdough and packaging/baking time. This study showed that the par-baking
conditions can be fine-tuned in order to increase the shelf-life of par-baked breads.

Acknowledgements

The authors wish to acknowledge the good cooperation with Luc De Clercq (Director of L’Atelier du Pain, Belgium) and L’Atelier du Pain (Ninove, Belgium) for providing sourdough and information on the production process.

References


