# Impact of process parameters on the specific volume of wholemeal wheat bread made using sourdough- and baker's yeast-based leavening strategies

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# Abstract

The final quality of wheat wholemeal bread is determined by the process parameter settings and leavening strategy. We hypothesise that the used leavening strategy may influence the optimal process parameter settings and, as such, the specific volume of the bread loaf. To analyse this interaction, bread was leavened with (*i*) a type 1 sourdough (SB), (*ii*) a type 1 sourdough combined with baker's yeast (YSB), or (*iii*) baker's yeast (YB). For each leavening strategy, the specific volume of bread, in response to variations in mixing time (4-10/4-14 min), water absorption (60-85%), and proofing time (1-7/1-3 h), was analysed using an I-optimal response surface experimental design. Data modelling identified a substantially lower maximal specific volume of SB (2.13 mL/g), compared to YSB (3.35 mL/g) and YB (3.26 mL/g). The proofing time and water absorption mostly influenced the specific volume of the SB and YSB, respectively. However, the mixing and proofing times mainly affected the specific volume of YB. The type 1 sourdough reduced the mixing time and water absorption required for an optimal specific volume of bread compared to baker's yeast. These results challenge the idea of yielding higher volumes upon using sourdough compared to baker's yeast and highlight the importance of optimisation of bread dough formulations and breadmaking processes.

**Sourdough, Bread volume, Whole grain, Leavening, Breadmaking, Triticum**

# Abbreviations

SB: Sourdough bread, leavened with a type 1 sourdough

YSB: Bread with sourdough, leavened with a type 1 sourdough and baker's yeast

YB: Yeasted bread, leavened with baker's yeast

LAB: Lactic acid bacteria

WA: Water absorption

$CO_2$: Carbon dioxide

dm: Dry matter

mc: Moisture content

DY: Dough yield

RR: Refreshment rate
TTA: Total titratable acidity
RH: Relative humidity
$R^2$: Coefficient of determination

# 1 Introduction

The consumption of whole-grain foods is an essential part of a healthy diet and sustainable lifestyle (Willett et al., 2019). According to the EAT-Lancet commission, at least 30% of the daily calories should originate from the consumption of whole grains (Willett et al., 2019). Consequently, the use of wholemeal wheat flour, having an extraction rate of 100% and hence consisting of all grain constituents, is receiving more attention (De Angelis et al., 2019; Ma et al., 2021). As bread is a staple food worldwide, the interest in producing high-quality wholemeal wheat bread products is rising (Cauvain, 2020). However, the specific volume, an essential bread quality attribute, remains low for yeast-leavened wholemeal wheat bread (Hemdane et al., 2016). Moreover, the specific volume could impact the nutritional aspects of bread loaves, such as the satiety index and glycaemic response (Burton and Lightowler, 2006). The addition of sourdough could improve the organoleptic quality of bread, as several researchers have established that the use of sourdough leads to a higher specific volume compared to a yeasted control (Clarke et al., 2002; Corsetti et al., 1998, 2000; Crowley et al., 2002; De Vuyst et al., 2021; Esteve et al., 1994; Ma et al., 2021).

Type 1 sourdough production relies on the spontaneous outgrowth of lactic acid bacteria (LAB) and yeasts in a mixture of flour and water (De Vuyst et al., 2017, 2021; Decock and Cappelle, 2005; Martín-Garcia et al., 2021). Throughout different backslopping steps, the microbial ecology of type 1 sourdough productions is shaped by physicochemical parameters, such as the consistency (dough yield), temperature, pH, fermentation time, and redox potential (De Vuyst et al., 2021; Martín-Garcia et al., 2021). These parameters lead to the natural selection of a characteristic microbiota that thrives in the unique environment of type 1 sourdoughs (Brandt, 2019; De Vuyst et al., 2021). These microorganisms produce organic acids during fermentation and their accumulation acidifies the environment (De Vuyst et al., 2021; Jayaram et al., 2013; Martín-Garcia et al., 2021). Carbon dioxide ($CO_2$) production by the yeasts and the heterofermentative LAB during fermentation of the flour-water mixture gives rise to an active sourdough, a gaseous bread dough, and finally, an airy bread (Arendt et al., 2007).

The volume of a bread loaf is determined by both the production and the retention of gas during the breadmaking process (Goesaert et al., 2005). The unique ability of the wheat dough to retain gas is mainly caused by the presence and functionality of the visco-elastic gluten network and the effect of water-extractable arabinoxylans (Campbell and Martin, 2020; Courtin and Delcour, 2002). However, the outcome of various descriptive studies on the effect of sourdough on bread volume differs from that of exploratory studies investigating the gluten network properties during sourdough production. In general, the addition of sourdough leads to the weakening and depolymerisation of this network due to the effect of acidification and the activity of various enzymes, which could decrease the gas retention capacity of the dough (Arendt et al., 2007; Gänzle et al., 2008; Goesaert et al., 2005; Takeda et al., 2001; Thiele et al., 2004; Xu et al., 2018). However, a vast body of literature describes enhanced loaf volumes when sourdough-type bread is compared to a yeasted control (Clarke et al., 2002; Corsetti et al., 1998, 2000; Crowley et al., 2002; De Vuyst et al., 2021; Esteve et al., 1994; Ma et al., 2021). It has been hypothesised that protein-related parameters affect bread volume to a lesser extent when sourdough is used in breadmaking (Thiele et al., 2004). Furthermore, the gas-holding capacity of the dough would be enhanced because of the increase of water-extractable arabinoxylans and the formation of exopolysaccharides during fermentation (De Vuyst et al., 2021; Thiele et al., 2004). Nevertheless, some studies report a decrease in bread volume upon the addition of sourdough (Armero and Collar, 1996; Rouzaud and Martínez-Anaya, 1997). This discrepancy could arise from different experimental breadmaking procedures that are used in research which are designed to check a hypothesis and may differ from the conditions used in industry, traditional bakeries or at the household level. First, many studies have compared sourdough and baker's yeast as leavening agents while keeping the process parameters, such as water absorption (WA), mixing time and proofing time, constant (Clarke et al., 2002; Corsetti et al., 1998, 2000; Crowley et al., 2002; De Angelis et al., 2019; Esteve et al., 1994; Komlenić et al., 2010; Salovaara and Valjakka, 1987; Thiele et al., 2002; Xu et al., 2018, 2019). Second, baker's yeast is commonly used together with sourdough to accelerate leavening during breadmaking. Consequently, the process does not solely rely on the leavening capacity of the sourdough microorganisms (Clarke et al., 2002; Crowley et al., 2002; De Angelis et al., 2019; Esteve et al., 1994; Garzon et al., 2021; Komlenić et al., 2010; Rouzaud and Martínez-Anaya, 1997; Salovaara and Valjakka, 1987; Thiele et al., 2002; Xu et al., 2018, 2019). Third, no standardised protocols for sourdough bread production exist, despite the large number of papers describing the effect of sourdough on the final bread quality. To date, to the best of our knowledge, no research has investigated and compared the impact of process parameters on the specific volume of wholemeal wheat bread made with and without sourdough.

We hypothesise that varying breadmaking processes and limited process optimisation may contribute to the inconsistency in the literature. This could lead to false conclusions concerning the effect of sourdough addition on the bread volume. Therefore, the aim of this study was to investigate and compare the effect of different leavening strategies on the process and the specific volume of wholemeal wheat bread. Hereto, three types of bread distinguished by the used leavening strategy were studied: *(i)* sourdough bread (SB), solely leavened with a type 1 sourdough; *(ii)* bread with sourdough (YSB), leavened with a combination of a type 1 sourdough and baker's yeast; and *(iii)* yeasted bread (YB), exclusively leavened with baker's yeast. For each leavening strategy, the analysis and modelling of the specific volume and crumb acidity of the bread in response to variations in mixing time, WA, and proofing time were aimed at, using response surface experiments with a prediction- and optimisation-oriented I-optimal experimental design. These models will generate novel insights into the interaction of the used leavening agent and the breadmaking process parameters, and their influence on the specific volume of the end product.

# 2 Materials and methods

## 2.1 Materials

Commercial wholemeal wheat flour without additives (Integral Cylindre, Ceres, Brussels, Belgium) [14.2% (m/m) moisture content (mc), 11.6% (m/m) dry matter (dm) protein, 1.60% (m/m) ash] was used to produce sourdough and bread dough. The moisture, protein (N x 5.7), and ash content were determined in triplicate according to AACC methods 44–15.02, 46-30.01, and AACC 08-01.01, respectively. For the latter, an elemental analyser-isotope ratio mass spectrometer (Erba EA1108 elemental analyser, Milan, Italy) was used. Vital wheat gluten was obtained from Cargill (Vilvoorde, Belgium). Salt and fresh compressed baker's yeast (Algist Bruggeman, Ghent, Belgium) were purchased from a local supermarket. Shortening (B&G Foods, Parsippany-troy Hills, NJ, USA) was used to lightly grease the baking tins and the working surface.

## 2.2 Sourdough production

A type 1 sourdough (200 g) with a dough yield [$\mathrm{DY} = (\mathrm{m_{flour}} + \mathrm{m_{water}}) * 100/\mathrm{m_{flour}}$ ] of 200 was made in a 400-mL glass beaker. On day 0, wholemeal wheat flour [100.00 g; 14% (m/m) mc] was added to 100.00 mL of tap water (23°C) and mechanically mixed to homogeneity for 1 min with a spiral mixer (Braun Multiquick 500 Watt, Kronberg, Germany). This mixture was covered and incubated at 30 °C for 24 h. From day 1 to day 10, the sourdough was refreshed with a refreshment rate (RR) of 10% (m/m). Given that 10% of the flour in the new mixture originated from the fermented flour-water mixture, 20.00 g of the incubated mixture was mixed with 90.00 g of wholemeal wheat flour and 90.00 mL of tap water. Hence, the water present in the preferment was also taken into account to keep the DY and the final volume constant. After ten days of daily refreshments and incubation, it was assumed that a stable ecosystem was obtained and the active, mature, type 1 sourdough could be further used as mother sourdough, which was stored at 4 °C to allow for less frequent refreshment steps (De Angelis et al., 2019; De Vuyst et al., 2017).

## 2.3 Sourdough storage and refreshment

Long-term sourdough storage was performed as follows. Every 7 days, a refreshment was performed to keep the microorganisms metabolically active. To this end, the cold mother sourdough was homogenised (30 s) and mixed with wholemeal wheat flour and tap water [10% (m/m) RR], as described above. The covered mixture was incubated at 30 °C until a pH of 4.0 was reached. The freshly fermented sourdough was subsequently stored at 4 °C and was considered the new mother sourdough.

## 2.4 Biochemical analysis of sourdough

The pH of the sourdough was analysed by inserting a pH probe (Hannah instruments, Temse, Belgium) directly into the sourdough. Using an automated titrator (Metrohm, Antwerp, Belgium), the total titratable acidity (TTA) of the sourdough was determined. Hereto, the amount (mL) of 0.10 M NaOH needed to reach a pH value of 8.5 in a homogenised mixture of 10.00 g of sourdough and 100.00 mL of deionised water was determined (Van der Meulen et al., 2007).

## 2.5 Sourdough activation

Before using sourdough as a leavening agent in breadmaking in small bakeries or at a household level, it is common to activate the stored mother sourdough. Activation steps were performed with a RR of 50% (m/m). Therefore, 100.00 g of mother dough was mixed with 50.00 g of wholemeal wheat flour and 50.00 mL of tap water. After mixing for 60 s to homogenise this mixture, it was covered and incubated until a pH of 4.0 was achieved. After two activation steps, the $CO_2$ production rate and acidification rate were stable (results not shown). After an overnight resting step (16 h at 4 °C), which did not significantly affect the activity in the dough, sourdough was included as a leavening agent or additive in the dough formulation of the SB and the YSB, respectively.

## 2.6 Culture-dependent microbiological analysis of sourdough

To enumerate and identify the microorganisms in the activated sourdough, a culture-dependent analysis was performed after its initial production and after one year of storage with weekly refreshments, as described previously (Comasio et al., 2020). Briefly, decimal dilutions of fresh sourdough were plated on modified de Man-Ragosa-Sharpe-5 (mMRS-5) agar medium (Harth et al., 2016), supplemented with 0.4 g/l of cycloheximide (Sigma-Aldrich, Saint-Louis, MO, USA) and 0.005 g/l of amphotericin B (Sigma-Aldrich), and on yeast extract-peptone-dextrose (YPG) agar medium, supplemented with 0.2 g/l of chloramphenicol (Sigma-Aldrich), to determine the colony forming units (CFU) per g of sourdough for LAB and yeasts, respectively. Plating was performed in triplicate and the plates were incubated at 30 °C for 48 h. To identify the microorganisms, 16 colonies were randomly picked from appropriate dilutions on the mMRS-5 and YPG agar media, transferred to 10 mL of mMRS-5 or YPG medium, and grown at 30 °C. After overnight incubation, 2 mL of culture was centrifuged, and the cell pellets obtained were used for DNA extraction, as described previously (Comasio et al., 2020). Purified genomic DNA was used to classify and identify the bacteria and yeasts by $(GTG)_5$-PCR and M13-PCR fingerprinting analysis, respectively, followed by numerical cluster analysis of the fingerprints obtained (Comasio et al., 2019, 2020). The species identity of each cluster was confirmed by sequencing the 16S rRNA gene (bacteria) or the internal transcribed spacer (ITS) region (yeasts).

## 2.7 Wholemeal wheat bread production

Three wholemeal wheat bread types were produced and distinguished based on the leavening agent: (*i*) sourdough bread (SB), leavened with a type 1 sourdough; (*ii*) bread with sourdough (YSB), for which a type 1 sourdough was combined with baker's yeast; and (*iii*) yeast-leavened bread (YB), for which only baker's yeast was added for leavening. The total mass of wholemeal wheat flour in the bread dough for all bread types was 100.00 g [14% (m/m) mc; 86.00 g dm]. Leavening was accomplished by including 20.00 g of activated sourdough in the bread doughs of SB and YSB and/or

2.00 g of baker's yeast in the bread doughs of YSB and YB. The flour in the sourdough accounted for 10% (m/m) of the total flour in the dough formulation for SB and YSB. In addition, 1.70 g of salt and 6.00 g of vital gluten were added before mixing (Table 1). The dough WA was varied and calculated as follows:

$$WA = x - (f - f_{14\%}) + w_s$$

with x (mL) the amount of water added to the dough formulation; $f_{14\%}$ (g) the theoretical flour mass (100 g); f (g) the actual flour mass, with an equivalent dm mass to the theoretical flour mass; and $w_s$ (g) the amount of water in sourdough added to the bread formulation.

The ingredients were mixed in a 100 g pin mixer bowl (National Manufacturing Lincoln, NE, USA). The WA was varied between 60 and 85% and the mixing time between 4 and 14 min (Table 2). The first fermentation lasted 15 min for YSB and YB and 120 min for SB. The final fermentation, further referred to as proofing, took place after sheeting and moulding in a lightly greased baking tin. Both first fermentation and proofing were performed in a fermentation cabinet (National Manufacturing) at 30 °C and relative humidity (RH) of 85%. The proofing time in the fermentation cabinet (30 °C, 85% RH) was varied (Table 2). All doughs were baked at 230 °C in a rotary oven for 24 min (National Manufacturing). The loaf volume was determined with a Volscan Profiler (Stable Micro Systems, Godalming, UK). The specific volume of the bread loaves was calculated by dividing the loaf volume (mL) by the loaf mass (g) 1 h after baking. The loaves were stored in the freezer (-18 °C) until further analysis. To assess the pH of the crumb, 10.00 g of the thawed crumb was homogenised in 100 mL of deionised water.

## 2.8 Experimental design of the bread making experiment

A response surface methodology, widely used in process optimisation, was applied to design the bread making experiment and analyse the resulting data. This method involves a quadratic regression model that approximates the relationship between the responses and the experimental factors, while testing each factor at three different levels. The aim was to understand and model the impact of WA (%), mixing time (min), and proofing time (h) on the specific loaf volume (mL/g) of each bread type (SB, YSB, and YB). The I-optimal designs showed no aliasing between the main effects and the second-order effects. Accordingly, they are orthogonal minimally aliased response surface (OMARS) designs (Núñez Ares and Goos, 2020; Núñez Ares et al., 2023). For each process, 30 loaves were baked over three days (random blocks). The experimental design for each bread type can be found in Supplementary Table 1. The range (Table 2) for each experimental factor was determined by means of preliminary tests, evaluating the dough handling, the bread volume, and the crumb cellular structure.

The model for each process was fitted using generalised least squares in combination with the restricted maximum likelihood method for the variances of the block effects and errors. The significant parameter estimates ($p < 0.05$) included in the model were determined with backward elimination and the models were evaluated using the coefficient of determination ($R^2$). The statistical analysis was conducted in the JMP Pro 16.0.0 software (SAS Institute, Cary, NC, USA). The estimated effect of the process parameters on the specific volume was visualised by the prediction profiler in the JMP software. Optimal process parameter values were estimated by optimising the desirability function embedded in the software. The specific volume of bread in the validation experiment was compared using one-way ANOVA and the Tukey multiple comparison procedure, after verifying that the specific volumes can be assumed to be normally distributed using a Shapiro-Wilk test and an Anderson-Darling test (Goos and Meintrup, 2016).

# 3 Results

## 3.1 Characterisation and activation of the type 1 sourdough

The viable counts of the sourdough were stable over one year and revealed the presence of 9.1 log (CFU/g) of LAB (mMRS-5 agar counts) and 7.4 log (CFU/g) of yeasts (YPG). The microbial composition of the type 1 sourdough initially produced consisted of the LAB species *Levilactobacillus brevis* and the yeast species *Saccharomyces cerevisiae*. However, the microbial community of this sourdough, weekly refreshed for one year, consisted of two LAB species, *Lactiplantibacillus plantarum* and *Levl. brevis,* together with the yeast species *S. cerevisiae*. The activation procedure led to a stable pH of 4.11 ± 0.03 and a TTA value of 12.83 ± 0.26 mL before the inclusion of the type 1 sourdough in the bread dough.

## 3.2 Effect of process parameters on the specific volume of wholemeal wheat bread produced with different leavening strategies

Three separate I-optimal response surface experiments were carried out to evaluate the effect of mixing time, WA, and proofing time on the specific volumes of the SB, YSB, and YB. The specific volume of the SB ranged between 1.36 and 2.23 mL/g. However, higher specific volumes were obtained for the YSB and YB, as those varied between 1.79-3.39 mL/g and 1.83-3.45 mL/g, respectively (Supplementary Table 1). Statistical analysis of these data led to models with high predictive values, as the $R^2$ was 0.90, 0.91, and 0.86 for the models of SB, YSB, and YB, respectively. In addition, the models revealed a low day-to-day and residual variance, resulting in a total variance of 0.0057, 0.0240, and 0.0272 $(mL/g)^2$ for the models of SB, YSB, and YB, respectively (Supplementary Table 2).

The relationship between the three process factors and the specific volume of bread, using the three different leavening strategies, revealed an optimum for most of the plots because of the significant quadratic effects of the process parameters (Figure 1). However, for the YB, the mixing time showed a linear relationship with the specific volume. According to the fitted response surface model, the SB approached a maximal specific volume of 2.13 mL/g when 7 min of mixing was combined with a WA of 67% and a proofing phase of 4 h and 11 min. Including the 2-h fermentation time applied in this process, the dough would spend a total time of 6 h and 11 min in the fermentation cabinet (30 °C, 85% RH) to obtain the maximal specific volume. According to the statistical analysis, the YSB had the potential to reach a maximal specific volume of 3.30 mL/g if the dough was mixed for 7 min and 30 s with a WA of 71%. After the 15-min fermentation time, 2 h and 11 min of proofing were needed to acquire the maximal specific loaf volume.

The estimated models for the SB and YSB both contained an intercept, the main effect, and the quadratic effect of the three tested parameters (Table 3, Supplementary Table 2). In addition, the interaction effect of mixing time and WA was significant in these models (Table 3). For the YB, the model consisted of the intercept and the main effect of the three parameters. However, only the quadratic effects of the proofing time and WA were significant in this model (Table 3). Additionally, the interaction effects of WA with both proofing and mixing time were significant (Table 3).

Given that the model for the YB involves a negative linear effect of the mixing time on the specific volume, the optimal mixing time was likely to be lower than the minimum value of 8 min used in the experiment. Therefore, ten extra tests were carried out to expand the design space for mixing to 4 min. To this end, an I-optimal follow-up experimental design was made with the JMP software (Supplementary Table 1). The model based on the combined data from the initial and the follow-up experiment contained all terms from the original model as well as a significant quadratic effect of the mixing time (Table 3). However, the model fit was slightly lower ($R^2 = 0.81$; Figure 2) than the original. This model indicated that the combination of 10 min of mixing, a WA of 74%, and a proofing time of 2 h and 15 min led to a maximal predicted specific volume of 3.26 mL/g for the YB.

Expressing the parameter estimates relative to the intercept revealed the most decisive parameter for each process (Table 3), which can also be visually deduced from the curve steepness in Figures 1 and 2. In addition, the relative parameter estimate values enabled the comparison of the relative influence of the process parameters across the models of the different processes. For the SB, the quadratic effect of proofing time ($P_{SB}^2$) influenced the model of the specific volume the most. The specific volume of the YSB was impacted the most influenced by the quadratic effect of WA ($WA_{YSB}^2$). In the process of the YB, the quadratic terms of both mixing time ($M_{YB}^2$) and proofing time ($P_{YB}^2$) made them the most prominent parameters influencing the specific volume. However, the difference in the impact of the three process parameters on the specific volume for the YB process was limited.

The models were validated by a confirmatory baking trial, testing the predicted optimal process parameter settings leading to the maximal specific volume (Table 4). The mean specific volume (n = 3) of the SB (1.96 ± 0.02 mL/g) was significantly lower than the mean specific volumes of the YSB and YB (3.32 ± 0.10 and 3.42 ± 0.01 mL/g, respectively).

### 3.3 Effect of leavening strategy and processing on the crumb acidity of wholemeal wheat bread

The crumb pH varied between 3.98 and 5.27 for the SB and between 5.01 and 5.63 for the YSB (Supplementary Table 1). In contrast, the crumb pH was higher for the YB, varying between 5.92 and 6.15. The effect of mixing time, WA, and proofing time on the pH of the crumb was modelled for the three leavening strategies based on the initial I-optimal design (n = 30; Supplementary Table 3). For bread containing type 1 sourdough (SB and YSB), both WA and proofing time had a significant influence on the crumb pH. These models involve the main, quadratic, as well as the interaction effect of the two factors, and showed a high predictive value ($R^2 = 0.99$). Figure 3 shows that proofing time had the most pronounced effect in both models. In contrast, the crumb pH of the YB was only affected by WA and had a lower goodness of fit ($R^2 = 0.87$). Filling in the process parameter settings that would lead to a maximal estimated specific volume in these models made it possible to predict the crumb pH when maximising the specific volume. This led to a predicted pH of 4.25, 5.30, and 6.02 for the crumb of the SB, YSB, and YB, respectively.

## 4 Discussion

As inconsistencies concerning the impact of sourdough on the specific volume of bread occur in the literature and may be ascribed to varying breadmaking processes and limited process optimisation applied, the present study examined the effect of the leavening strategy (type 1 sourdough, baker's yeast, or a combination thereof) on the process parameters and specific volume of wholemeal wheat bread. Therefore, a modelling approach was applied. The combination of the high predictive value of the different models and the low unexplained variance for each response surface experiment indicated that a suitable set of factors was examined. In addition, the predictive power was confirmed by a baking trial testing the predicted process parameter settings, leading to the maximal specific volume of bread. Therefore, mixing time, WA, and proofing time proved to be important factors in steering the specific volume of bread leavened with a type 1 sourdough, baker's yeast, or with their combination, when the process was performed at a constant temperature. Furthermore, a sourdough with constant activity during breadmaking was obtained after the activation procedure, as the pH and TTA of the sourdough were stable and limited day-to-day variation was detected during the experiments. Identification of the microbiota of the Type 1 sourdough of the present study revealed the occurrence of Levilactobacillus *brevis*, *Lactiplantibacillus plantarum*, and *Saccharomyces cerevisiae*. The fact that, initially, *Levl. brevis* and *S. cerevisiae* occurred as the sole microorganisms, followed by the additional presence of *Lacp. plantarum,* after one year, indicated that sourdough is a dynamic environment in which lactic acid bacteria and yeasts can evolve over time (De Vuyst et al., 2017, 2021). These dynamics need to be ascribed to the number of backsloppings, the duration of the fermentation step, the temperature of the fermentation and storage steps, etc. The selection of *Levl. brevis* may be ascribed to the low storage temperature of the sourdoughs in between the weekly backsloppings (Vancanneyt et al., 2006, Liu et al., 2020, Zhang et al., 2021). However, the microbial stability of sourdoughs considered over a long period has seldom been studied (Bessmeltseva et al., 2014). Yet, *Levl. brevis, Lacp. plantarum*, and *S. cerevisiae* belong to the most reported LAB and yeast species in sourdough (Van Kerrebroeck et al., 2017; Arora et al., 2021; De Vuyst et al., 2023). Although *Frul. sanfranciscensis* is frequently reported in bakery sourdoughs, it requires adapted backslopping regimes and fermentation and storage durations and temperatures. The follow-up of the LAB and yeast dynamics, both prevailing and background species, can be performed both culture-dependently (present study) and culture-independently (e.g., PCR amplicon-based high-throughput sequencing and metagenomics), both techniques encompassing several biases (Calabrese et al., 2022). However, culture-independent techniques allow an in-depth microbiological characterisation (Weckx et al., 2019, Comasio et al., 2020, Landis et al., 2021, Calabrese et al., 2022), whereas culture-dependent techniques usually focus on the most abundant species.

As the optimal WA to produce the SB (67%) and the YSB (71%) was lower than for the YB (74%), the addition of type 1 sourdough lowered the amount of water needed to maximise the specific bread volume. A similar result was found with farinograph experiments with the addition of pure organic acids or sourdough prepared with a starter culture of *Levl. brevis* (Clarke et al., 2002; Komlenić et al., 2010; Maher Galal et al., 1978). The WA had a pronounced influence on the bread loaf specific volume of all bread types. However, it was most decisive in the short procedures of YSB and YB ($WA_{SB}^2 = -3.3\%$, $WA_{YSB}^2 = -14.1\ \%$, $WA_{YB}^2 = -7.4\ \%$).

Comparing the mixing time leading to the maximal estimated specific volume of bread for different leavening strategies revealed a shorter mixing time when a type 1 sourdough was used. This was in line with earlier studies that reported a reduced optimal mixing time, determined with farinograph experiments, for more acidic doughs (Jayaram et al., 2014; Maher Galal et al., 1978; Wehrle et al., 1997). Interestingly, the larger quadratic effect of mixing time in the model of the specific volume of YB ($M_{YB}^2 = -10.4\%$), compared to SB and YSB ($M_{SB}^2 = -5.4\%$, $M_{YSB}^2 = -5.8\%$), indicated that deviations in mixing time had a larger influence on the specific volume of YB within the analysed design space. A possible explanation for the reduced mixing time could be found in the effect of the lower dough pH because of the addition of sourdough. Apart from an increase in the electrostatic repulsion between the gluten, the formation of free thiolate anion ($S^-$) groups may be reduced (Clarke et al., 2004; Delcour et al., 2012; Jayaram et al., 2014; Maher Galal et al., 1978; Rombouts et al., 2012; Schober et al., 2003). These negatively charged cysteine residues are required to execute the nucleophilic attack on a sulphur atom, leading to new intermolecular disulfide bonds during mixing. This reaction occurs less under acidic conditions because the $pK_a$ value of cysteine is approximately 8.5 (Delcour et al., 2012; Rombouts et al., 2012), so fewer intermolecular disulfide bonds can be formed. In addition, the glutathione reductase activity of the heterofermentative *Levl. brevis* during fermentation may stimulate thiol/disulphide interchange reactions between glutathione and gluten. This may lead to the depolymerisation of the glutenin macropolymer during mixing (Xu et al., 2018). Given the above, it can be assumed that the dough gluten network reaches the optimal consistency faster upon adding this type 1 sourdough. Still, the resulting gluten network in the dough could be softer and less cohesive.

The proposed models indicated that proofing time had a substantial influence on the specific volume of bread for the three leavening strategies tested. However, the relative impact compared to WA and mixing time differed. When leavening occurred solely with the described microbial consortium in the type 1 sourdough used, the proofing time needed to be prolonged (4 h 11 min) and this parameter had the largest impact of the three experimental factors on the loaf specific volume ($P_{SB}^2$ = -14.7%). In contrast, the models of the specific volume of the YSB and YB suggested shorter optimal proofing times (2 h 11 min and 2 h 15 min, respectively) and showed a smaller impact of the proofing time ($P_{YSB}^2$ = -6.6% and $P_{yb}^2$ = -9.7%) in the model outcome compared to WA ($WA_{YSB}^2$ = -14.7%) or mixing time ($M_{YB}^2$ = -10.4%), respectively. This was in line with the expectations, as it is known that in sourdough breadmaking, prolonged fermentation processes are commonly used (Martín-Garcia et al., 2021). In addition, baker's yeast is widely used in the breadmaking industry for its fast and strong $CO_2$ production capacity (Struyf et al., 2017).

The acidity of the crumb was highly correlated with the proofing time when sourdough was used. The pronounced acidification due to the combination of sourdough and the prolonged proofing time could lead to an increased protease activity and weakened dough integrity and, accordingly, a lower gas retention capacity of the dough (Bleukx et al., 1997; Clarke et al., 2004; Schober et al., 2003; Su et al., 2019). When over-proofing occurred, the dough weakening led to a collapse of the structure during proofing and baking (results not shown). Small bread loaf volumes when the crumb pH decreased below 5.0 have also been reported before (Crowley et al., 2002). However, more research is needed to understand the underlying mechanisms.

The results of the present study suggested that there was no practically meaningful difference in maximal specific volume between the YSB (3.32 ± 0.10 mL/g) and the YB (3.42 ± 0.01 mL/g). However, the SB (1.96 ± 0.02 mL/g) showed a substantially smaller maximal specific volume than the YSB and YB. The same trend was found when the volume (mL) was analysed (results not shown), indicating that the weight effect of higher WA levels did not interfere with the outcome. The limited effect of sourdough, produced with a defined hetero- and homofermentative strain and used as an additive, on the specific volume of YSB (3.18 ± 0.06 mL/g and 3.32 ± 0.06 mL/g with *Fructilactobacillus sanfranciscensis* DSM 20451 and *Latilactobacillus sakei* LS8, respectively) compared to YB (3.18 ± 0.04 mL/g), has been reported before (Xu et al., 2018). The observations in the present study did not support the idea that adding sourdough improved the specific volume of wholemeal wheat bread. No improvement compared to the use of baker's yeast was established when a type 1 sourdough, containing a microbial consortium of *Levl. brevis, Lacp. plantarum*, and *S. cerevisiae*, was used as the only leavening agent as well as when it was used combined with baker's yeast. The physicochemical changes in the protein network that are linked to the addition of sourdough did not appear to enlarge the specific volume. This inconsistency with previous research (Clarke et al., 2002; Crowley et al., 2002; Xu et al., 2019) could be attributed to the limited process parameter optimisation for the yeasted control bread in previous research. This idea was supported by the models developed within this study to analyse the effect of leavening strategies on the process parameters and the specific volume of wholemeal wheat bread. The mixing time influenced the specific volume of the YB the most, and it turned out that a longer mixing time was more beneficial for the volume of the YB than for that of the YSB. Therefore, this study revealed that, when the process parameter settings of a breadmaking experiment are optimised for the use of sourdough, the experiment will not be able to achieve the optimal specific volume of the yeasted control bread.

It has previously been described that dough rheological properties, acidification rate, and bread volume are influenced by the strains present in a sourdough (Corsetti et al., 1998; Esteve et al., 1994). Although this study only evaluated the effect of one type 1 sourdough, the insights gained from this study may be of assistance in investigating breadmaking procedures using other sourdoughs as well. If the described effects of acidification were responsible for the decrease in gas retention during breadmaking, it is believed that the results can be extrapolated for sourdough fermented wheat bread that is strongly acidified. However, the mechanisms contributing to the impact of acidity on specific volumes are not fully understood. In addition, the results of this study could not predict the outcome of consortia with specific attributes such as a high $CO_2$ production rate or exopolysaccharide production.

The current data highlighted the importance of process optimisation while studying and comparing volume-related quality aspects of bread. This counts for quality aspects both from an organoleptic and nutritional point of view. Furthermore, this work highlighted that limited process optimisation could explain part of the inconsistencies found in the literature that describes the effect of sourdough in breadmaking. The outcome of this study challenged the idea of bread volume improvement simply by using sourdough instead of baker’s yeast, as sourdough did not improve the specific volume of wholemeal wheat bread. More research using optimised breadmaking experiments is needed to reveal the impact of dough acidification by sourdough fermentation during prolonged breadmaking processes on the gas retention capacity of the dough and, as such, on the final bread volume.

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## Conflict of interest

The authors declare that no commercial or financial relationships were conflicting with the research, and that, as such, there is no conflict of interest.

# 5 Tables and figures

Table 1. Dough formulation of sourdough bread, bread with sourdough, and yeasted bread. Flour mass is expressed on theoretical flour moisture basis (14%), however, the actual flour mass was adjusted to take the deviation in moisture content due to sourdough addition into account. The fermented flour in sourdough (DY = 200) was taken into account to keep the final dry matter mass of flour in the different dough formulations constant. Three theoretical levels of water absorption (WA) were analysed and adjusted for the flour's moisture content deviation. As such, the actual water absorption of bread doughs with the same theoretical water absorption, made with different processes, was constant.

| ***Bread dough ingredients*** | **Sourdough bread (SB)** | **Bread with sourdough (YSB)** | **Yeasted bread (YB)** |
|---|---|---|---|
| *Flour (g)* | 90.00 | 90.00 | 100.00 |
| *Salt (g)* | 1.70 | 1.70 | 1.70 |
| *Gluten (g)* | 6.00 | 6.00 | 6.00 |
| *Sourdough (g)* | 20.00* | 20.00* | - |
| *Yeast (g)* | - | 2.00 | 2.00 |
| *Water (mL)* | | | |
| *WA of 60%* | 50.00 | 50.00 | 60.00 |
| *WA of 72.5%* | 62.50 | 62.50 | 72.50 |
| *WA of 85%* | 75.00 | 75.00 | 85.00 |

*10 g of fermented flour present in 20 g of sourdough

Table 2. Experimental design to analyse the impact of process parameters on the specific loaf volume of sourdough bread, bread with sourdough, and yeasted bread. The number of runs (N), random blocks, and tested factor levels for the I-optimal experimental design are shown in the first column. The ranges for water absorption, mixing time, and proofing time were determined based on preliminary experiments.

| ***I-optimal response surface experiment*** | **Sourdough bread (SB)** | **Bread with sourdough (YSB)** | **Yeasted bread (YB)** |
|---|---|---|---|
| *N* | 30 | 30 | 30 |
| *Random blocks* | 3 | 3 | 3 |
| *Water absorption (%)* | 60.0 - 72.5 - 85.0 | 60.0 - 72.5 - 85.0 | 60.0 - 72.5 - 85.0 |
| *Mixing time (min)** | 4 - 7 – 10 | 4 - 7 – 10 | 8 - 11 - 14 |
| *Proofing time (h)** | 1 - 4 - 7 | 1 - 2 - 3 | 1 - 2 - 3 |

*Time (min, h) had an accuracy of 1 s.

Table 3. Parameter estimates of the models for sourdough bread, bread with sourdough, and yeasted bread. Expressing estimate values relative to the value of the intercept (%) makes it possible to compare the importance of the factor between the models for different breadmaking processes, describing the impact of process parameters on the specific volume of bread.

**Sourdough bread (SB)**

| Significant effects | Estimates | Relative to intercept (%) |
|---|---|---|
| Intercept | 2.11 | |
| Proofing time*Proofing time ($P_{SB}^2$) | - 0.31 | - 14.73 |
| Mixing time*Mixing time ($M_{SB}^2$) | - 0.11 | - 5.41 |
| Mixing time*Water absorption | 0.08 | 3.89 |
| Water absorption*Water absorption ($WA_{SB}^2$) | - 0.07 | - 3.27 |
| Water absorption (60-85%) | - 0.06 | - 3.04 |
| Mixing time (4-10 min) | 0.04 | 1.96 |
| Proofing time (1-7 h) | 0.04 | 1.79 |

**Bread with sourdough (YSB)**

| Significant effects | Estimates | Relative to intercept (%) |
|---|---|---|
| Intercept | 3.28 | |
| Water absorption*Water absorption ($WA_{YSB}^2$) | - 0.46 | - 14.15 |
| Mixing time*Water absorption | 0.30 | 9.30 |
| Proofing time*Proofing time ($P_{YSB}^2$) | - 0.22 | - 6.64 |
| Mixing time*Mixing time ($M_{YSB}^2$) | - 0.19 | - 5.85 |
| Water absorption (60-85%) | - 0.17 | - 5.29 |
| Mixing time (4-10 min) | 0.10 | 3.03 |
| Proofing time (1-3 h) | 0.06 | 1.89 |

**Yeasted bread (YB)**
**follow-up**

| Significant effects | Estimates | Relative to intercept (%) |
|---|---|---|
| Intercept | 3.24 | |
| Mixing time*Mixing time ($M_{YB}^2$) | - 0.34 | - 10.41 |
| Proofing time*Proofing time ($P_{YB}^2$) | - 0.31 | - 9.70 |
| Water absorption*Mixing time | 0.29 | 9.08 |
| Water absorption*Water absorption ($WA_{YB}^2$) | - 0.24 | - 7.38 |
| Proofing time (1-3 h) | 0.13 | 4.00 |
| Water absorption*Proofing time | 0.10 | 3.10 |
| Mixing time (4-14 min) | 0.09 | 2.64 |
| Water absorption (60-85%) | 0.01 | 0.31 |

Table 4. Validation of the models for the specific loaf volume of sourdough bread, bread with sourdough, and yeasted bread. The predicted process parameter settings leading to the optimal specific volume of bread are listed in combination with the corresponding estimated specific volume. The average (n = 3) specific volume and a picture of the crumb of the bread loaves are shown. Different letters indicate a significant difference for the specific volume tested using one-way ANOVA and the Tukey multiple comparison procedure ($p < 0.05$).

| Model validation | Sourdough bread (SB) | Bread with sourdough (YSB)- | Yeasted bread (YB) |
|---|---|---|---|
| Mixing time | 7 min | 7 min 30 sec | 10 min |
| WA | 67% | 71.20% | 74% |
| Proofing time | 4 h 11 min | 2 h 11 min | 2 h 15 min |
| Estimated volume (mL/g) | [ 2.07 - 2.18 ] | [ 3.16 - 3.44 ] | [ 3.19 - 3.50 ] |
| Specific volume (mL/g) | $1.96^{A}$ ± 0.02 | $3.32^{B}$ ± 0.10 | $3.42^{B}$ ± 0.01 |
| Crumb picture |  |  |  |

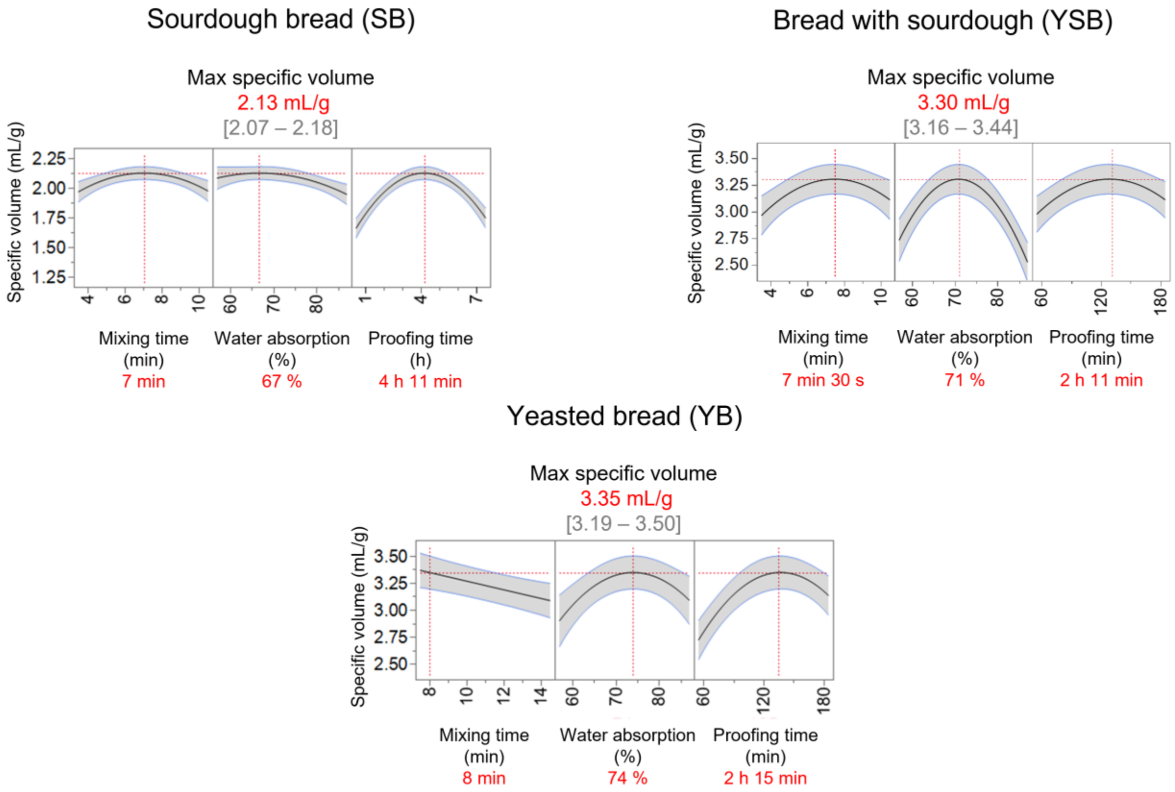

Figure 1. Graphical representation of the final response surface model for the specific volume of bread as a function of the process parameters of sourdough bread, bread with sourdough, and yeasted bread. The black line indicates the model estimation, whereas the grey area demonstrates the uncertainty. Values for the process parameter settings, leading to the maximal estimated specific loaf volume, are noted under the graphs and are predicted by using the prediction profiler in

the statistic software. The resulting maximal estimated specific volume and the confidence interval for the model outcome are given above the graphs.

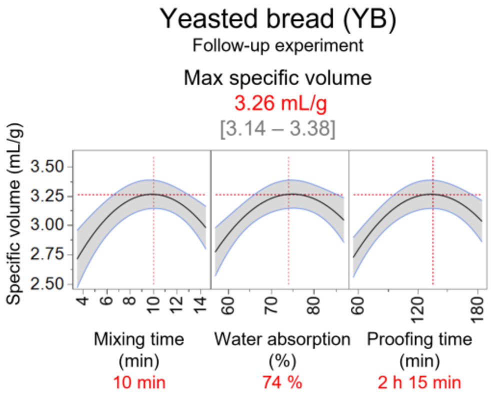

Figure 2. Graphical representation of the final response surface model, based on the combined data from the original and follow-up experiments, estimating the specific volume of yeasted bread. The black line indicates the model, whereas the grey area demonstrates the uncertainty. Values for the process parameter settings, leading to the maximal estimated specific loaf volume, are noted under the graph and are predicted by using the prediction profiler in the statistic software. The resulting maximal estimated specific volume and the confidence interval for the model outcome are given above the graph.

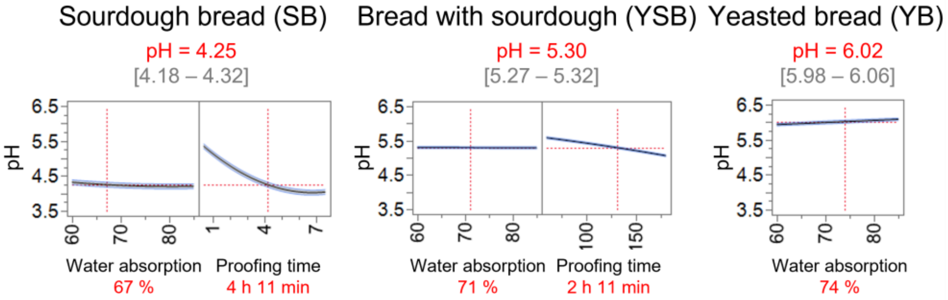

Figure 3. Graphical representation of the final response surface models describing the acidity of wheat wholemeal bread crumbs as a function of the process parameters of sourdough bread, bread with sourdough, and yeasted bread. The black line indicates the model estimation, whereas the grey area demonstrates the uncertainty. The process parameter values leading to the maximal specific loaf volume are inserted in the model to estimate the crumb acidity when these conditions are applied. Confidence intervals for the predicted pH are given between square brackets above the graphs.

## Supplementary tables

Supplementary Table 1. I-optimal design of the experiments to investigate the effect of process parameters on the specific volume and crumb pH of sourdough bread, bread with sourdough, and yeasted bread, along with the runs that are carried out within the I-optimal follow-up experiment of yeasted bread.

| **Sourdough bread (SB)** | | | | | | |
|---|---|---|---|---|---|---|
| Run | Random block | Mixing time (min) | Water absorption (%) | Proofing time (h) | Specific volume (mL/g) | pH |
| 1 | 1 | 7 | 72.5 | 7 | 1.78 | 4.02 |
| 2 | 1 | 10 | 60.0 | 1 | 1.64 | 5.24 |
| 3 | 1 | 4 | 72.5 | 4 | 1.97 | 4.32 |
| 4 | 1 | 4 | 85.0 | 1 | 1.36 | 5.23 |
| 5 | 1 | 7 | 60.0 | 1 | 1.79 | 5.27 |
| 6 | 1 | 4 | 60.0 | 7 | 1.76 | 4.10 |
| 7 | 1 | 10 | 85.0 | 4 | 2.00 | 4.26 |
| 8 | 1 | 7 | 72.5 | 4 | 2.04 | 4.25 |
| 9 | 1 | 7 | 85.0 | 4 | 2.10 | 4.21 |
| 10 | 1 | 10 | 72.5 | 7 | 1.68 | 3.99 |
| 11 | 2 | 4 | 60.0 | 4 | 1.96 | 4.43 |
| 12 | 2 | 10 | 85.0 | 1 | 1.58 | 5.11 |
| 13 | 2 | 10 | 72.5 | 4 | 2.12 | 4.27 |
| 14 | 2 | 10 | 60.0 | 4 | 1.95 | 4.37 |
| 15 | 2 | 7 | 60.0 | 7 | 1.87 | 4.09 |
| 16 | 2 | 7 | 72.5 | 1 | 1.75 | 5.09 |
| 17 | 2 | 7 | 72.5 | 4 | 2.23 | 4.26 |
| 18 | 2 | 4 | 85.0 | 7 | 1.56 | 3.99 |
| 19 | 2 | 4 | 72.5 | 1 | 1.68 | 5.06 |
| 20 | 2 | 7 | 85.0 | 7 | 1.64 | 3.99 |
| 21 | 3 | 4 | 60.0 | 1 | 1.73 | 5.16 |
| 22 | 3 | 10 | 60.0 | 7 | 1.69 | 4.01 |
| 23 | 3 | 7 | 72.5 | 4 | 2.13 | 4.22 |
| 24 | 3 | 7 | 85.0 | 1 | 1.61 | 5.02 |
| 25 | 3 | 7 | 60.0 | 4 | 2.02 | 4.31 |
| 26 | 3 | 10 | 72.5 | 1 | 1.62 | 5.11 |
| 27 | 3 | 4 | 72.5 | 7 | 1.70 | 4.02 |
| 28 | 3 | 10 | 85.0 | 7 | 1.78 | 3.98 |
| 29 | 3 | 7 | 72.5 | 4 | 2.08 | 4.21 |
| 30 | 3 | 4 | 85.0 | 4 | 1.61 | 4.16 |

Supplementary Table 1. - continued

| **Bread with sourdough (YSB)** | | | | | | |
|---|---|---|---|---|---|---|
| Run | Random block | Mixing time (min) | Water absorption (%) | Proofing time (min) | Specific volume (mL/g) | pH |
| 1 | 1 | 10 | 85.0 | 60 | 2.54 | 5.59 |
| 2 | 1 | 7 | 85.0 | 120 | 2.52 | 5.34 |
| 3 | 1 | 10 | 72.5 | 180 | 3.09 | 4.97 |
| 4 | 1 | 10 | 60.0 | 120 | 2.57 | 5.34 |
| 5 | 1 | 4 | 60.0 | 60 | 2.35 | 5.58 |
| 6 | 1 | 4 | 85.0 | 180 | 1.79 | 5.01 |
| 7 | 1 | 7 | 60.0 | 180 | 2.73 | 5.09 |
| 8 | 1 | 4 | 72.5 | 120 | 3.13 | 5.32 |
| 9 | 1 | 7 | 72.5 | 60 | 2.81 | 5.60 |
| 10 | 1 | 7 | 72.5 | 120 | 3.35 | 5.35 |
| 11 | 2 | 10 | 85.0 | 180 | 2.77 | 5.03 |
| 12 | 2 | 7 | 72.5 | 180 | 3.10 | 5.05 |
| 13 | 2 | 10 | 72.5 | 120 | 3.07 | 5.35 |
| 14 | 2 | 4 | 85.0 | 60 | 1.90 | 5.65 |
| 15 | 2 | 7 | 60.0 | 120 | 3.01 | 5.34 |
| 16 | 2 | 4 | 60.0 | 120 | 3.04 | 5.33 |
| 17 | 2 | 7 | 72.5 | 120 | 3.39 | 5.35 |
| 18 | 2 | 7 | 85.0 | 60 | 2.49 | 5.62 |
| 19 | 2 | 4 | 72.5 | 180 | 2.94 | 5.08 |
| 20 | 2 | 10 | 60.0 | 60 | 2.53 | 5.51 |
| 21 | 3 | 4 | 85.0 | 120 | 1.89 | 5.38 |
| 22 | 3 | 10 | 85.0 | 120 | 2.96 | 5.36 |
| 23 | 3 | 7 | 60.0 | 60 | 2.91 | 5.54 |
| 24 | 3 | 4 | 72.5 | 60 | 2.78 | 5.60 |
| 25 | 3 | 4 | 60.0 | 180 | 2.99 | 5.12 |
| 26 | 3 | 10 | 72.5 | 60 | 2.74 | 5.63 |
| 27 | 3 | 7 | 72.5 | 120 | 3.29 | 5.31 |
| 28 | 3 | 7 | 85.0 | 180 | 2.46 | 5.04 |
| 29 | 3 | 7 | 72.5 | 120 | 3.20 | 5.37 |
| 30 | 3 | 10 | 60.0 | 180 | 2.32 | 5.13 |

Supplementary Table 1. - continued

| | | | **Yeasted bread (YB)** | | | |
|---|---|---|---|---|---|---|
| Run | Random block | Mixing time (min) | Water absorption (%) | Proofing time (min) | Specific volume (mL/g) | pH |
| 1 | 1 | 11 | 72.5 | 60 | 2.56 | 5.99 |
| 2 | 1 | 11 | 85.0 | 120 | 2.99 | 6.05 |
| 3 | 1 | 14 | 60.0 | 60 | 2.40 | 5.95 |
| 4 | 1 | 11 | 85.0 | 180 | 3.15 | 6.05 |
| 5 | 1 | 8 | 72.5 | 120 | 3.36 | 6.02 |
| 6 | 1 | 8 | 72.5 | 60 | 2.79 | 5.99 |
| 7 | 1 | 14 | 60.0 | 180 | 2.35 | 5.92 |
| 8 | 1 | 8 | 60.0 | 180 | 2.83 | 5.92 |
| 9 | 1 | 14 | 85.0 | 120 | 3.26 | 6.10 |
| 10 | 1 | 11 | 72.5 | 120 | 3.17 | 6.03 |
| 11 | 2 | 14 | 72.5 | 180 | 2.77 | 5.96 |
| 12 | 2 | 11 | 72.5 | 180 | 2.98 | 5.98 |
| 13 | 2 | 8 | 60.0 | 120 | 2.87 | 5.91 |
| 14 | 2 | 14 | 72.5 | 120 | 3.01 | 6.01 |
| 15 | 2 | 11 | 60.0 | 60 | 2.56 | 5.98 |
| 16 | 2 | 8 | 85.0 | 180 | 2.80 | 6.06 |
| 17 | 2 | 11 | 60.0 | 120 | 2.71 | 5.90 |
| 18 | 2 | 14 | 85.0 | 60 | 2.33 | 6.09 |
| 19 | 2 | 11 | 72.5 | 120 | 3.31 | * |
| 20 | 2 | 8 | 85.0 | 60 | 2.26 | 6.08 |
| 21 | 3 | 8 | 60.0 | 60 | 2.74 | 5.93 |
| 22 | 3 | 11 | 60.0 | 180 | 2.43 | 5.94 |
| 23 | 3 | 11 | 72.5 | 120 | 3.21 | 6.01 |
| 24 | 3 | 8 | 85.0 | 120 | 3.45 | 6.08 |
| 25 | 3 | 11 | 85.0 | 60 | 2.51 | 6.12 |
| 26 | 3 | 11 | 72.5 | 120 | 3.15 | 6.00 |
| 27 | 3 | 14 | 85.0 | 180 | 3.08 | 6.15 |
| 28 | 3 | 14 | 72.5 | 60 | 2.54 | 6.05 |
| 29 | 3 | 8 | 72.5 | 180 | 3.37 | 6.05 |
| 30 | 3 | 14 | 60.0 | 120 | 2.29 | 5.96 |
| 31 | 4 | 4 | 72.5 | 120 | 2.72 | |
| 32 | 4 | 4 | 85.0 | 180 | 1.83 | |
| 33 | 4 | 4 | 85.0 | 60 | 1.91 | |
| 34 | 4 | 4 | 60.0 | 180 | 2.61 | |
| 35 | 4 | 4 | 60.0 | 60 | 2.54 | |
| 36 | 4 | 11.5 | 72.5 | 60 | 2.69 | |
| 37 | 4 | 11 | 60.0 | 120 | 2.68 | |
| 38 | 4 | 12 | 72.5 | 120 | 3.11 | |
| 39 | 4 | 12 | 85.0 | 180 | 2.77 | |
| 40 | 4 | 11 | 72.5 | 120 | 3.24 | |

[*] No data available

Supplementary Table 2. Model estimation for the specific volume of sourdough bread, bread with sourdough, and yeasted bread. In addition, the model, based on the combined data from the original and follow-up experiments, that estimates the specific volume of yeasted bread is given. Parameter estimates, standard error, and probability of the significant effects ($p < 0.05$) are shown. The summary of fit and variance levels are a measure of the model quality and process control of the I-optimal designed experiments.

**Sourdough bread (SB)**

| Significant effects | Estimates | Standard error | Prob >\|t\| |
|---|---|---|---|
| Intercept | 2.11 | 0.03 | < 0.0001 |
| Proofing time*Proofing time | - 0.31 | 0.03 | < 0.0001 |
| Mixing time*Mixing time | - 0.11 | 0.03 | 0.0008 |
| Mixing time*Water absorption | 0.08 | 0.02 | 0.0012 |
| Water absorption*Water absorption | - 0.07 | 0.03 | 0.0263 |
| Water absorption (60-85%) | - 0.06 | 0.02 | 0.0017 |
| Mixing time (4-10 min) | 0.04 | 0.02 | 0.0301 |
| Proofing (1-7 h) | 0.04 | 0.02 | 0.0457 |
| Summary of fit | | $R^2$ | 0.90 |
| | | $R^2$ Adj | 0.87 |
| Variance | | Block | - 0.0002 |
| | | Residual | 0.0057 |
| | | Total | 0.0057 |

**Bread with sourdough (YSB)**

| Significant effects | Estimates | Standard error | Prob >\|t\| |
|---|---|---|---|
| Intercept | 3.28 | 0.06 | < 0.0001 |
| Water absorption*Water absorption | - 0.46 | 0.06 | < 0.0001 |
| Mixing time*Water absorption | 0.30 | 0.04 | < 0.0001 |
| Proofing time*Proofing time | - 0.22 | 0.06 | 0.0009 |
| Mixing time*Mixing time | - 0.19 | 0.06 | 0.0027 |
| Water absorption (60-85%) | - 0.17 | 0.03 | < 0.0001 |
| Mixing time (4-10 min) | 0.10 | 0.03 | 0.0093 |
| Proofing time (60-180 min) | 0.06 | 0.03 | 0.0879 |
| Summary of fit | | $R^2$ | 0.91 |
| | | $R^2$ Adj | 0.89 |
| Variance | | Block | 0.0026 |
| | | Residual | 0.0214 |
| | | Total | 0.0240 |

**Yeasted bread (YB)**

| Significant effects | Estimates | Standard error | Prob >\|t\| |
|---|---|---|---|
| Intercept | 3.18 | 0.06 | < 0.0001 |
| Proofing time*Proofing time | - 0.33 | 0.06 | < 0.0001 |
| Water absorption*Water absorption | - 0.24 | 0.06 | 0.0006 |
| Proofing time (60-180 min) | 0.17 | 0.04 | 0.0002 |
| Water absorption*Proofing time | 0.17 | 0.05 | 0.0014 |
| Water absorption (60-85%) | 0.15 | 0.04 | 0.0007 |
| Mixing time (8-14 min) | - 0.14 | 0.04 | 0.0016 |
| Water absorption*Mixing time | 0.13 | 0.05 | 0.0097 |
| Summary of fit | | $R^2$ | 0.86 |
| | | $R^2$ Adj | 0.82 |
| Variance | | Block | 0.0025 |
| | | Residual | 0.0247 |
| | | Total | 0.0272 |

**Yeasted bread (YB)**
**Follow-up**

| Significant effects | Estimates | Standard error | Prob >\|t\| |
|---|---|---|---|
| Intercept | 3.24 | 0.06 | < 0.0001 |
| Mixing time*Mixing time | - 0.34 | 0.07 | < 0.0001 |
| Proofing time*Proofing time | - 0.31 | 0.07 | < 0.0001 |
| Water absorption*Mixing time | 0.29 | 0.06 | < 0.0001 |
| Water absorption*Water absorption | - 0.24 | 0.07 | 0.0009 |
| Proofing time (60-180 min) | 0.13 | 0.04 | 0.0025 |
| Water absorption*Proofing time | 0.10 | 0.05 | 0.0395 |
| Mixing time (4-14 min) | 0.09 | 0.05 | 0.0976 |
| Water absorption (60-85 %) | 0.01 | 0.04 | 0.8062 |
| Summary of fit | | $R^2$ | 0.81 |
| | | $R^2$ Adj | 0.76 |

Supplementary Table 3. Model estimation for the crumb pH of sourdough bread, bread with sourdough, and yeasted bread. Parameter estimates, standard error, and probability of the significant effects ($p < 0.05$) are shown. The summary of fit and variance levels are a measure for the model quality and process control of the I-optimal designed experiments.

**Sourdough bread (SB)**

| Significant effects | Estimates | Standard error | Prob >\|t\| |
|---|---|---|---|
| Intercept | 4.25 | 0.02 | < 0.0001 |
| Proofing time (1-7 h) | - 0.56 | 0.01 | < 0.0001 |
| Proofing time*Proofing time | 0.30 | 0.02 | < 0.0001 |
| Water absorption (60-85%) | - 0.06 | 0.01 | < 0.0001 |
| Water absorption *Water absorption | 0.04 | 0.02 | 0.01 |
| Summary of fit | | $R^2$ | 0.99 |
| | | $R^2$ Adj | 0.99 |
| Variance | | Block | 0.0011 |
| | | Residual | 0.0018 |
| | | Total | 0.0028 |

**Bread with sourdough (YSB)**

| Significant effects | Estimates | Standard error | Prob >\|t\| |
|---|---|---|---|
| Intercept | 5.35 | 0.01 | < 0.0001 |
| Proofing time (1-7 h) | - 0.27 | 0.01 | < 0.0001 |
| Water absorption*Proofing time | - 0.04 | 0.01 | < 0.0001 |
| Proofing time*Proofing time | - 0.02 | 0.01 | 0.05 |
| Water absorption (60-85%) | 0.00 | 0.01 | 0.73 |
| Summary of fit | | $R^2$ | 0.99 |
| | | $R^2$ Adj | 0.98 |
| Variance | | Block | 0.0001 |
| | | Residual | 0.0007 |
| | | Total | 0.0009 |

**Yeasted bread (YB)**

| Significant effects | Estimates | Standard error | Prob >\|t\| |
|---|---|---|---|
| Intercept | 6.01 | 0.01 | < 0.0001 |
| Water absorption (60-85%) | 0.08 | 0.01 | < 0.0001 |
| Summary of fit | | $R^2$ | 0.89 |
| | | $R^2$ Adj | 0.88 |
| Variance | | Block | 0.0002 |
| | | Residual | 0.0006 |
| | | Total | 0.0009 |

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