

The Effects of Water Content and Weight of Inoculum on the Production of Sourdough

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Abstract

The use of sourdough has ancestral origin but could be useful too in modern baking to obtain unique breads, with enhanced sensorial properties, stability and nutritional benefits. We study the process of obtaining sourdoughs from whole wheat flour, in different condition of moisture and refreshments, at 25°C and 24 hours of fermentation for 21 refreshments. We observed that after 2 weeks all sourdoughs showed a stabilisation of final pH and TTA, the wettest one stabilised after 1 week. The more consistent sourdough had a higher final TTA (22 mL NaOH 1N / 100 g) than the more fluid ones (22 mL NaOH 1N / 100 g) but the yield of acids reported to dry matter were similar, more favourable for the more fluid sourdough. The sourdough with a flour: water ratio 1:2 showed a very good ratio lactic acid:acetic acid, 7:1. Changing the refreshment ratio from 1:3 to 1:4 did not have noticeable effects.

Keywords: sourdough, lactobacilli, wheat flour, refreshment ratio

1. Introduction

The modern breadmaking technology use mixers with a high energy input and high amounts of yeast (*Saccharomyces cerevisiae*) to obtain quick and cheap breads with a high specific volume. This kind of bread usually has a bland taste and is more and more often rejected by consumer. New market opportunities appear for bakers able to produce traditional breads which imply the use of sourdough and other products as corn, potato, rye flour. Still the modern bread plant could combine tradition with modern technology to obtained high quality breads with low costs, high specific volume, less additives and improved taste, small and taste. One method is the use of sourdough. Many sourdoughs are commercially available [1] but these could be obtained locally, with a small investment. The self-obtained sourdough could have the advantage of distinct product on the market. The sourdough technology is very old and

simple [2], which is almost forgotten. The commercially available sourdoughs are type II and III [2, 3] and are standardised, obtained with selected microorganisms (lactic acid bacteria – LAB and yeasts). Traditions could be combined in modern baking with efficiency and traditional like sourdough could be obtained in bakery plants. The obtaining of sourdough is very simple and implies mixing the flour and water in different proportions and incubating at specific temperature for a time, usually for 24h, followed by periodical refreshments [1]. During the consecutive refreshment LAB and yeast species presents in flour are selected, cultivated and propagate through refreshment. It is necessary several weeks for the development of a stable microflora. In the first days of refreshment prevail bacteria from the genera *Enterococcus* and *Lactococcus*, in the following days the genera *Lactobacillus*, *Pediococcus*, and *Weissella* replaced the first ones. After first week LAB species from the genera *Lactobacillus* prevailed and the yeast number

reach a peak [3] In the traditional sourdoughs, type I [2], the obligat heterofermentative lactobacilli prevail over the homofermentative lactobacilli [4]. The LAB species presents in dough are *Lb. sanfranciscensis*, *Lb. alimentarius*, *Lb. brevis*, *Lactococcus lactis*, subsp. *lactis*, *Lb. fermentum*, *Leuconostoc citreum* [5]. The most common yeast species present in sourdoughs were *Candida humilis*, *Saccharomyces cerevisiae*, *Saccharomyces exiguous*, *Candida holmii*, *Saccharomyces minor*, *Kluyveromyces marxianus*, *Torulasporea delbrueckii* [6, 7, 8]. The LAB and yeast population of sourdough could be very stable over a long period of time, for years or even for decades [1].

The sourdough are used to improve the sensorial properties of breads, taste, smell and aroma. These effects are proved through many researches [2, 5, 9, 10, 11, 12] but other beneficial effects were observed. The use of sourdough reduces the incidences of rope disease [13, 14, 15] and fungal spoilage [16, 17, 18, 19]. Other important aspects of sourdough are improvement of dough rheology [2, 10, 20, 21], antistaling effect [22, 23, 24, 25] and even nutritional benefits [6, 26, 27, 28].

The aim of this study is to monitorise the process of sourdough formation to find how many refreshment are necessary to obtain stable sourdoughs in different condition. We choose the incubation temperature 25°C to promote the LAB species present in traditional sourdoughs, obtained at medium temperature, not at high temperature specific for plant scale. We varied the flour:water ratio from 1:0.6 to 1:3. The refreshment ratio was 1 part fermented doughs to 3 parts of fresh flour-water slurry. For the flour:water ratio 1:2 we use also the refreshment ratio 1:4.

2. Material and Methods

For the sourdough preparation was used commercial whole wheat flour available on market and tap water to reproduce home conditions. Flour and water were mixed in different ratio, flour:water 1:0.6; 1:1; 1:2 and 1:3. The inoculum of lactic acid bacteria and wild yeast consisted in sourdough fermented for 24h. The proportion was 3 parts fresh dough and 1 part fermented dough. For the sourdoughs prepared from 2 part of flour and 1 part of water was prepared a sourdough with

1 part fermented dough for 4 parts fresh dough. The fermentation was conducted at 25°C. For 3 weeks the doughs were refreshed every day.

The pH was measured immediate after preparation and after 24h of fermentation with an electrode HANNA INSTRUMENTS FC200B, designed for semisolid food by direct immersion in dough. The total titratable acidity (TTA) was determined by titration with NaOH, 0.1N until pH 8.5 and after 10 min resting the titration was continued until final pH 8.5 stable for 1 min was reached. The pH was measured with pH electrode.

The lactic and acetic acids from sourdough were determined by HPLC, SmartLine Knauer 5000 equipped with autosampler Knauer 3950, degassing system Smartline Knauer 5000, pump Smartline Knauer 1000 and UV-VIS detector Smartline Knauer 2550. Mobile phase used was monosodium phosphate 100mM with pH adjusted to 2.5 with phosphoric acid. We used chromatographic column Macherey-Nagel EC Nucleodur 100-5 C18ec, 250 mm length and 4 mm in diameter. The mobile phase flow with 1 mL/min and the injection of sample was 5µL, as described by manufacturer of column, the temperature oven was 25°C. Detection was made spectrophotometric, at 210 nm. The acetic acid, lactic acid for calibration and other chemicals was purchased from SIGMA ALDRICH, HPLC grade.

3. Results and Discussion

The major aim of this study was to observed how evolve the sourdoughs during preparation according with their formulations, how long time is necessary to prepare a stable sourdough which could be used in bakery with a constant and reliable results. For this the dough refreshed daily where analysed for three weeks.

A very quick and simple method to monitorise the sourdough is by measuring the pH. In figure 1 are Presented the pH of the dough prepared freshly, day 0 is the day when the first mixture of flour and water was prepared. The water content of mixture influenced the pH, the pH was lower for the higher amount of water added. The higher water content probably influenced the dissociation of acids from flour. The initial pH of mixture fresh prepared dropped sharply in the first days and after first week we observed a stabilisation of pH.

A greater variance was observed for the pH of fermented doughs (Figure 2). After first 24 hours of fermentation the pH drops from an average initial 6.1 to 5.9 . A small acidification was observed in first day but in the following day the pH dropped under 4.0 . The slower acidification was observed for the dough with a higher ratio of flour, where higher amount of dry components

buffered the acids formed by LAB. The higher variance of pH was observed in the first 6 days of refreshment and in the last days the pH was almost constant. The doughs with a higher content of water had a lower pH due the higher rate of dissociation and lower quantity of substance to buffer the organic acids formed by LAB.

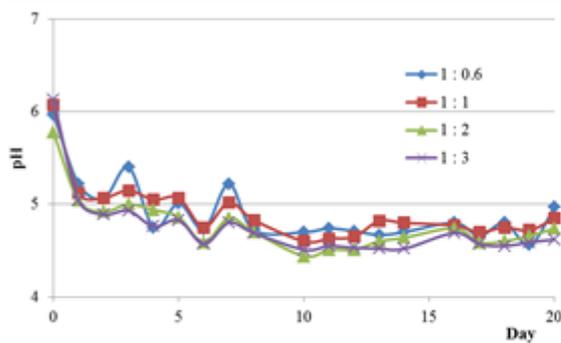


Figure 1. The initial pH of refreshed sourdoughs with different flour:water ratio

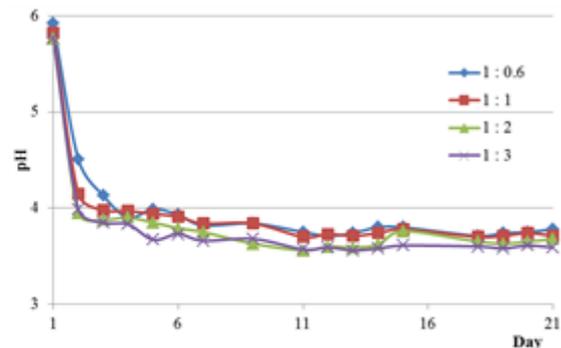


Figure 2. The final pH of sourdoughs with different flour:water ratio

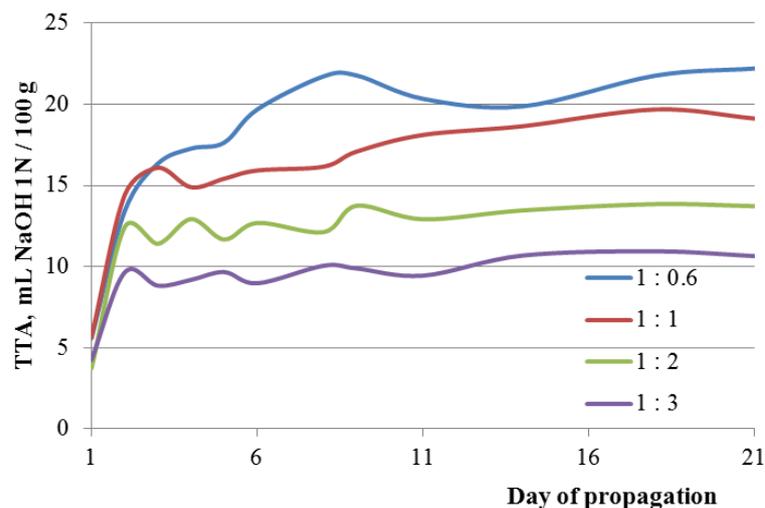


Figure 3. The TTA of fermented sourdoughs with different flour:water ratio

The sourdough's pH is influenced by different components of flour, proteins and mineral salts. Measuring the TTA is a more accurate to look at the changes which occur in doughs during fermentation. The values of sourdough's total acidity are presented in figure 3. The Final pH of dough did not dropped under 3.5, value which seemed to restrict the development of the LAB.

After the first day the acidities of doughs vary around the 5 mL NaOH 1N / 100g. The doughs with higher amount of water had a lower acidity by the dilution effect. The doughs with a higher amount of water showed more rapidly stabilisation of the acidity. The TTA of dough with a ratio flour:water of 1:3 reach 10 mL NaOH 1N / 100g and after that, during the rest of 3 weeks of refreshment vary a little around this value.

Those values were in accordance with the variation of pH. For the doughs with a higher amount of flour 1:0.6 and 1:1 the stabilisation did not occurred until the finish of the experiment, in the last days could be observed also a slight ascendant tendency of TTA. When we compared the TTA of these doughs with the corresponding pH we observed that the pH presented a stabilisation after the first third of experiment while the TTA continued to rise until the end of experiment. Another observation was made, the final pH of dough varied between 3.6 and 3.8 while the TTA varied much more, between 10.6 and 22.3 mL NaOH 1N / 100 g of dough.

We were interested about the yield of acids formed during fermentation in these different conditions of moisture. We calculated the TTA/100 g dry matter (d.m.) and the results were presented in figure 4. We observed that despite de TTA content of sourdough varied widely according with moisture content the TTA dry basis did not varied very much. The higher content of TTA dry basis was observed for the doughs with higher water content, which show a higher yield of acids. The lower water content inhibited the formation of acids by inhibiting microorganisms present in dough or by inhibiting the process of starch braking to maltose or both of these.

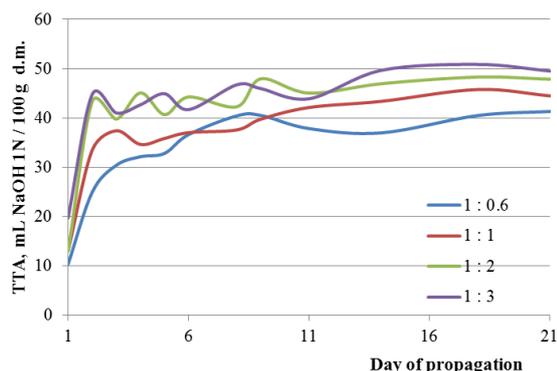


Figure 4. The TTA dry basis of fermented sourdoughs with different flour:water ratio

The TTA of dough is consisted from the natural occurring acid species of flour and the acids forms during fermentation. The most important species of acids formed during fermentation are lactic and acetic acid. The amounts of these acids from doughs were determined through HPLC analysis.

The lactic and acetic concentration in the doughs prepared with equal amount of water and flour were presented in figure 5.

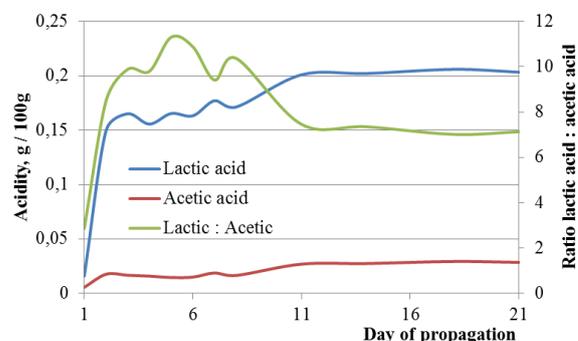


Figure 5. Lactic acid and acetic acid concentrations n dough prepared with equal amount of flour and water

Lactic acid and acetic acid were formed by LAB and wild yeast present in flour and propagated during daily refreshment of dough. Both species of microorganisms are able to form acetic acid while lactic acid is formed only by LAB. In the first half of experiment the concentration of lactic and acetic acids increases continuous, as the TTA, and in the last half a stabilisation occurred, and the concentrations of lactic and acetic acids were around 0.2 and 0.03 g/100 g of dough. These values are in accordance with values obtained by Lefebvre et al. [29] The ratio between lactic and acetic acid is important in bakery because a ratio lower than 4 means a sharp and more pregnant taste and smell [30]. In the first days of propagation we observed a high ratio favourable to lactic acid, even higher than 10. In the last days monitored the ratio fall to 7.3, still a very good. During propagation the yeast probably developed more and contributed to acetic acid formed during fermentation. The stabilisation indicated that a stable population of LAB and yeast developed in sourdough.

The refreshment ratio also could have importance in the process of sourdough formation. For the dough with a flour:water ratio 1:2 we work with two refreshment ratio (old dough: fresh dough ratio) 1:3 and 1:4. Practically no differences were observed between the two sourdoughs, excepting the initial pH of freshly prepared sourdough. As expected, the initial pH of sourdough with higher refreshment ratio was higher due the higher amount of acids carried through initial inoculum, but the final pH was very similar. At the beginning of sourdough formation the

final TTA of doughs with refreshment ratio 1:4 were slightly lower, probably due the lower number of LAB present in fresh prepared dough but in the last part of experiment the final TTA was higher. The high initial pH allowed higher starch degradation to nutrients for LAB and yeast presents doughs.

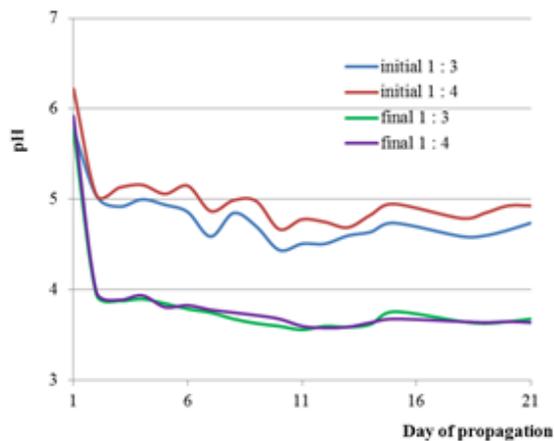


Figure 6. The initial and final pH of sourdoughs with 1:2 flour:water ratio and different refreshment ratio

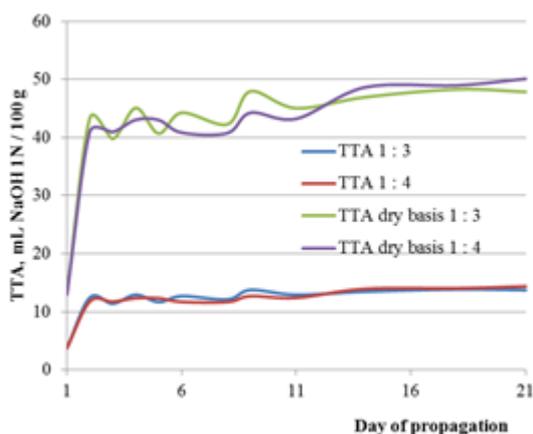


Figure 7. The TTA and TTA dry basis of fermented sourdoughs with 1:2 flour:water ratio and different refreshment ratio

4. Conclusions

We consider that the dough with a ratio of flour:water of 1:1 is more preferable in an industrial scale bakery because the dough is pumpable and a sedimentation did not occurred, as in the case of more diluted dough. The dough with a flour:water ratio 1:0.6 was very consistent and

sticky at the end of fermentation and difficult to work with high quantity. Higher amount of water lead to a less viscous dough which show sedimentation of flour's particles and need mixing to keep it in suspension.

The sourdoughs prepared at 25°C showed a stabilisation after 21 refreshments, the doughs with high water content showed a more rapid stabilisation than the dough with a high dry matter content. The high content of dry matter in sourdough lead to a higher acidification but the yield of acid formation expressed as TTA dry basis was more favourable to the dough with higher water content.

For a high yield of lactic acid in dough it is important to keep the initial pH low for starch degradation through endogenous amylases of flour. These can be realised by reducing the amount of inoculum used for refreshment or by increasing the water content. The high yield of acid could be achieved through the use of different salt with buffer properties as in modern technology or by the predigestion of flour-water slurry before inoculation, as in old fashioned bread technology [31].

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Compliance with Ethics Requirements. Authors declare that they respect the journal's ethics requirements. Authors declare that they have no conflict of interest and all procedures involving human / or animal subjects (if exist) respect the specific regulation and standards.

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