



DOI: 10.22144/ctu.jen.2021.002

Establishment of a spontaneously started sourdough in Vietnam

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Article info.

Received 30 Oct 2019

Revised 22 Apr 2020

Accepted 31 Mar 2021

Keywords

Bread flour, *Lactobacillus plantarum*, *Saccharomyces cerevisiae*, sourdough, *Wickerhamomyces anomalus*

ABSTRACT

The use of sourdoughs in bread baking in Vietnam has recently been increased but little is known about microorganisms in those sourdoughs. This study was to assess changes in pH value and microbial density of a sourdough from bread flour (BF) and a sourdough from all-purpose flour (AF) during propagation. The results showed that the type of flour did not cause a significant difference in pH changes, but it could contribute to the distinct levels of lactic acid bacteria (LAB) and yeast in the two sourdoughs. The BF sourdough gained proper maturation in 15 days when it reached pH of 3.69, 1.3×10^9 CFU g⁻¹ LAB and 7.4×10^8 CFU g⁻¹ yeast. Meanwhile, the AF sourdough had lower levels of LAB and yeast (3.9×10^8 CFU g⁻¹ and 1.0×10^8 CFU g⁻¹, respectively). Sequencing analysis revealed the presence of *Lactobacillus plantarum* in the BF sourdough on the 10th and 15th days of the propagation process. *Wickerhamomyces anomalus* was found on the 10th day while *Saccharomyces cerevisiae* was detected on the 15th day. This sourdough can be used in further studies to assess the benefits of sourdough in bread baking.

1. INTRODUCTION

The benefits of sourdough in improvement of bread texture, flavor and prolonged shelf life have long been demonstrated. Many studies have confirmed that these benefits are attributed to the presence and metabolic activities of lactic acid bacteria (LAB) and yeasts in sourdoughs (Esteve et al., 1994; Arendt et al., 2007; Coda et al., 2011; Black et al., 2013; Galle, 2013; Denkova et al., 2014).

Sourdough microbiota varies greatly depending on many factors (e.g., flour and other ingredients, environmental microbiota, dough hydration and yield, propagation steps, propagation time and temperature, and the use of starter culture or baker's yeast) (Scheirlinck et al., 2007; Minervini et al., 2012; Huys et al., 2013). It has been reported that the LAB

population in most spontaneously started sourdoughs reaches an equilibrium through a three-stage process featured by the prevalence of sourdough-atypical species (e.g., *Enterococcus* spp. and *Lactococcus lactis* subsp. *lactis*), sourdough-typical species (e.g., species of *Lactobacillus*, *Leuconostoc*, *Pediococcus*, and *Weissella*) and highly adapted sourdough-typical species (e.g., *Lb. fermentum* and *Lb. plantarum*) (Van Der Meulen et al., 2007; Weckx et al., 2010; Huys et al., 2013). Amongst the LAB species identified in sourdoughs, obligately heterofermentative lactobacilli dominate over the others (Huys et al., 2013). Sharing the complex sourdough ecosystem with these LAB species is the yeast population. A diversity of yeast species have been identified in sourdough including those of the genera *Saccharomyces*, *Candida* and *Wickerhamomyces* (Meroth et al., 2003; Minervini et al., 2012;

Huys et al., 2013). Many studies have shown that the LAB:yeast ratio usually reaches a range from 10:1 to 100:1 in mature sourdoughs (Gobbetti, 1998; Scheirlinck et al., 2007). It has also been shown that sourdough maturation is usually achieved within about 10 days (Van Der Meulen et al., 2007; Weckx et al., 2010).

Sourdough has been introduced to Vietnamese families by Western cultures in recent years but its use has yet been brought to a commercial scale. This is mainly due to the limitation of information on the microbial ecology of sourdoughs made in Vietnam.

In this study, bread flour and all-purpose flour were used to propagate two types of sourdough. The pH value of each type as well as LAB and yeast numbers were determined on the 5th, 10th and 15th days of the propagation process. Sequencing analysis was used to identify the microbial species in the more prevalent sourdough.

2. RESULTS AND DISCUSSION

The composition of the bread flour (BF) and the all-purpose flour (AF) used in sourdough propagation is listed in Table 1. These two types of flour are very similar except for the higher protein content in the bread flour.

Table 1. Composition (%) of two types of flours

Composition	Bread flour (BF)	All-purpose flour (AF)
Protein (%)	14.38	10.62
Total sugar (%)	3.35	3.42
Reducing sugar (%)	1.78	1.81
Total fat (%)	≤ 3	≤ 3

For both types of sourdough, pH values dropped rapidly in the first five days but remained almost unchanged from the 5th day through the 15th day (Fig. 1). This is in accordance with previous reports on pH changes of most types of sourdough due to production of organic acids by sourdough LAB (Van Der Meulen et al., 2007; Corsetti, 2013; Bessmeltseva et al., 2014; Harth et al., 2018). The data also showed that pH was not affected by flour type. The final pH values of the BF sourdough (ca. 3.69) and the AF sourdough (ca. 3.66) were both in the range of a well-developed sourdough (Esteve et al., 1994). However, the BF sourdough had a better bread leavening capacity. This is attributable to the

higher protein content in the bread flour than in the all-purpose flour (Table 1).

Lactic acid bacteria (LAB) population of the BF sourdough showed a continuous increase over the propagation period while that of the AF sourdough showed a decrease after 10 days (Fig. 2). Bacterial count of the former reached ca. 1.3×10^9 CFU g⁻¹ which is a common level found in mature sourdough (Meroth et al., 2003; Scheirlinck et al., 2007; Van Der Meulen et al., 2007; Ercolini et al., 2013; Bessmeltseva et al., 2014). On the other hand, the final number of LAB in the latter was significantly lower ($p < 0.05$), but it was in the range reported in some studies (Meroth et al., 2003; Scheirlinck et al., 2007; Van Der Meulen et al., 2007). LAB population in most sourdoughs has been reported to evolve through a three-stage process in which its number rises rapidly at first and stays constant after reaching a peak (Van Der Meulen et al., 2007; Weckx et al., 2010; Huys et al., 2013). The decrease of LAB number in the AF sourdough in this study did not show the same trend. This could be due to lower amount of nitrogen source in this type of flour (Table 1) (Scheirlinck et al., 2007; Minervini et al., 2012; Huys et al., 2013). In most of the previous studies, bread flour (high protein content) and high dough yields were used rather than all-purpose flour and low dough yields. The propagation time required for the BF sourdough in this study to reach the common number of LAB was longer than that in other reports, which could also be attributed to the properties of the flour and the low dough yield.

Meanwhile, the yeast count of the BF sourdough rose significantly from the 5th day to the 10th day although it remained almost the same level on the 15th day (Fig. 3). This level reached ca. 7.4×10^8 CFU g⁻¹, making the LAB-yeast ratio about 2:1. Although a ratio of 100:1 is more typical to sourdough, higher ratios of yeast were also found in some studies (Scheirlinck et al., 2007; Van Der Meulen et al., 2007). On the contrary, the yeast number of the all-purpose sourdough showed a gradual decrease over the experiment period (Fig. 3). It reached ca. 1.0×10^8 CFU g⁻¹ on the 15th day, making the LAB-yeast ratio about 4:1, again suggesting inadequate nitrogen source in the all-purpose flour. These differences between the two sourdoughs could explain the better bread leavening capacity of the BF sourdough although further studies are necessary.

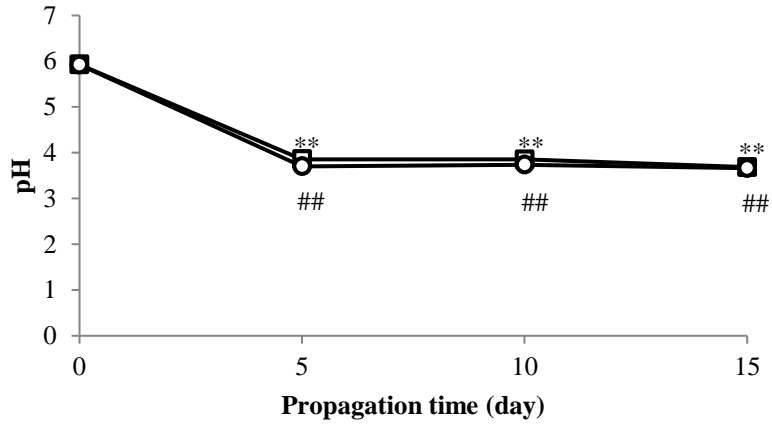


Fig. 1. pH of BF (□) and AF (○) sourdoughs

The data are the means of three independent experiments \pm SD (n = 3). **P < 0.01 compared with pH of BF sourdough on the initiation day, ##P < 0.01

compared with pH of AF sourdough on the initiation day by LSD post-hoc test.

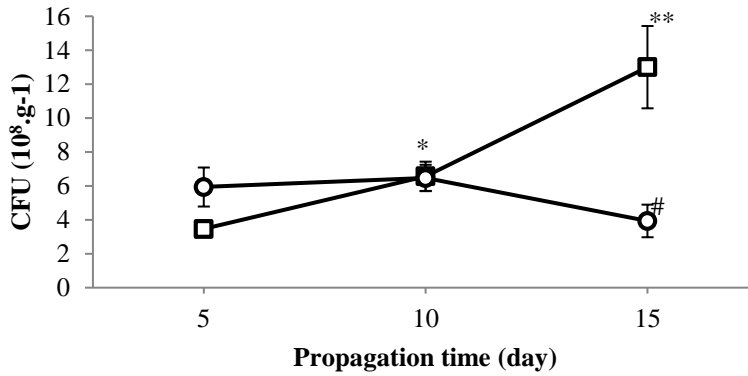


Fig. 2. Lactic acid bacteria cell numbers of BF (□) and AF (○) sourdoughs

The data are the means of three independent experiments \pm SD (n = 3). **P < 0.01, *P < 0.05 compared with LAB cell number of BF sourdough on the 5th

day, #P < 0.05 compared with LAB cell number of AF sourdough on the 5th day by LSD post-hoc test.

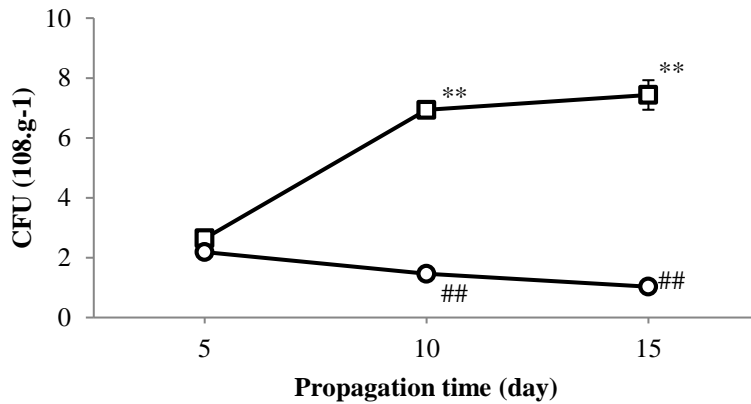


Fig. 3: Yeast cell numbers of BF (□) and AF (○) sourdoughs

The data are the means of three independent experiments ± SD (n = 3). **P < 0.01 compared with yeast cell number of BF sourdough on the 5th day, ##P < 0.01 compared with yeast cell number of AF sourdough on the 5th day by LSD post-hoc test.

Lactic acid bacteria and yeasts in the BF sourdough on the 10th day and the 15th day were isolated and identified (Table 2). *Lactobacillus plantarum* was present in the sourdough regardless of the propagation time. According to the literature, this LAB species was commonly found in both spontaneously started sourdoughs and culture-initiated sourdoughs (Scheirlinck et al., 2007; Van Der Meulen et al.,

2007; Weckx et al., 2010; Minervini et al., 2012; Huys et al., 2013). It has also been suggested that this species is one of the highly adapted sourdough-typical LAB species (Van Der Meulen et al., 2007; Weckx et al., 2010). Its presence could be detected in both the second stage and the third stage of the propagation process. Although the number of LAB in the BF sourdough was not as high as in other studies, the persistence of *L. plantarum* suggested that this sourdough reached maturation. As for the origin of the LAB, it has been recorded that *L. plantarum* adapts to sourdoughs from flour and the environment (Huys et al., 2013).

Table 2. Sequencing results

Sourdough	Closest relative	GenBank accession no.
Bread flour, the 10 th day	<i>Lactobacillus plantarum</i> strain KC28 chromosome (100% identity)	CP026743.1
Bread flour, the 10 th day	<i>Wickerhamomyces anomalus</i> (99% identity)	LC120363.1
Bread flour, the 15 th day	<i>Lactobacillus plantarum</i> strain RI-113 (99% identity)	CP017406.1
Bread flour, the 15 th day	<i>Saccharomyces cerevisiae</i> strain CEN.PK113-7D chromosome XII (99% identity)	CP022977.1

Regarding yeasts, *Wickerhamomyces anomalus* was identified on the 10th day, and *Saccharomyces cerevisiae* was identified on the 15th day in the BF sourdough. Both of these species were found repeatedly in most types of sourdough (Meroth et al., 2003; Ercolini et al., 2013; Huys et al., 2013). The sourdough on the 15th day showed better bread leavening capacity than it did on the 10th day while the yeast number did not change markedly, suggesting that the type of yeast played an important role. *S. cerevisiae* is well known for bread leavening capacity, hence the name baker’s yeast. *W. anomalus*, on the other hand, has not been used for bread baking except for sourdough bread. However, since we only examined one species for each time point of the propagation process, it remained to be investigated whether these species were the dominant yeasts at the respective time point. It has been suggested that both *S. cerevisiae* and *W. anomalus* in sourdoughs are from flour origin (Huys et al., 2013).

ACKNOWLEDGEMENTS

We thank An T. Nguyen and Chau T. K. Tran, Faculty of Food Technology and Biotechnology, Can Tho University of Technology, for their technical assistance.

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