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A low pH does not determine the community dynamics of spontaneously developed backslopped liquid wheat sourdoughs but does influence their metabolite kinetics

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ABSTRACT

This study dealt with the influence of a crucial pH value of 4.0 on the microbiota of spontaneously fermented backslopped liquid wheat sourdoughs. Two spontaneously fermented wheat sourdough fermentation experiments were carried out, one without control of the pH and one with the pH kept constant at pH 4.0, both during nine backslopping steps. In each case, two additional backslopping steps were carried out, with the pH kept constant at 4.0 and with free pH, respectively. Keeping the pH constant at 4.0 changed the microbial community dynamics and metabolite kinetics of the sourdough fermentations. A slower prevalence of sourdough-specific *Kazachstania* yeasts occurred. Nevertheless, in both experiments, *Lactobacillus fermentum*, *Lb. plantarum*/*pentosus*/*paraplantarum*, and *Kazachstania exigua*/*bulderi*/*barnettii* prevailed ultimately. The lactic acid and ethanol concentration profiles were affected positively by keeping the pH constant at a minimum of 4.0 as well as the L- and D-lactic acid ratio profile, a potential biological marker for sourdough stability and maturity. Also, the concentration and diversity of acetate esters and their precursors, in particular isoamyl acetate and isoamyl alcohol, were affected negatively by the pH control, indicating the role of pH stress in the sourdough aroma formation.

Keywords:
Wheat sourdough
pH evolution
Community dynamics
Lactic acid
Ethanol
Ester

1. Introduction

Sourdough is a mixture of cereal flour and water, to which other ingredients can be added, that is fermented to a low pH (Corsetti and Settanni, 2007; De Vuyst et al., 2009, 2014; Gobbetti and Gänzle, 2013; Gobbetti et al., 2014; Minervini et al., 2014). Due to the metabolic action of mainly yeasts and lactic acid bacteria (LAB), the fermentation mixture develops characteristics that are highly desirable from a technological, nutritional, and commercial point of view (Decock and Cappelle, 2005; Gänzle and Gobbetti, 2013). The microbiota can develop spontaneously, originating from the flour and/or other ingredients, or can be added as a starter culture (De Vuyst et al., 2014). Major metabolic activities of the microbial communities are acidification (LAB), leavening (yeasts and heterofermentative LAB), and flavor formation (yeasts and LAB). Further, breads made with sourdough are characterized by altered technological characteristics, such as texture improvement, delayed staling, and increased preservation (Gänzle, 2014; Gobbetti et al., 2014). Additional improved organoleptic and nutritional properties may result from the production of minor metabolites, such as diacetyl, acetoin, higher alcohols, aldehydes, and esters (yeasts and/or LAB); the degradation of peptides and liberation of amino acids; and the degradation of compounds such as phytate and subsequent improved access to nutrients (Gänzle, 2014; Gänzle et al., 2007, 2008; Gobbetti et al., 2005). Finally, the desire of consumers for authentic, tradition-based, healthy, and clean-label products contributes to a renewed, positive image of sourdough-based baked products (Decock and Cappelle, 2005; Gobbetti et al., 2014).

The sourdough ecosystem is a very specific microbial ecosystem, characterized not only by a low pH but also a variable carbohydrate

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content and nutrient and oxygen limitation (De Vuyst et al., 2014). Although glucose, fructose, and sometimes sucrose can be present in variable concentrations, the main carbohydrate source is maltose (Gobbetti et al., 1994). In the case of spontaneously matured wheat sourdoughs, whether or not backslopped, the microbiota develops into a consortium, often encompassing one to several LAB species and one or two yeast species (Huys et al., 2013; De Vuyst et al., 2014). For instance, wheat sourdoughs, spontaneously fermented under laboratory conditions are dominated by *Lactobacillus fermentum* and *Lactobacillus plantarum* upon backslopping (Van der Meulen et al., 2007; Minervini et al., 2010; Vrancken et al., 2011b). A complicated interplay between endogenous factors, such as flour enzymes, the ash content of the flour, the microbiota present, encompassing but not limited to LAB and yeasts, and exogenous factors, such as the process parameters imposed (temperature, dough yield, backslopping time, etc.), influence the microorganisms prevailing in the sourdough ecosystem, selecting for the species and strains most adapted. Yet, the exact influence of many of these factors is unknown (Hammes et al., 2005; Katina et al., 2004; Minervini et al., 2014). It has for instance been shown that one of the major factors and also one of the most difficult to assess is the pH (Gänzle et al., 1998; Guerzoni et al., 2007, 2013; Vrancken et al., 2011b). The sourdough pH is a result of the interplaying factors mentioned above. The pH of a sourdough is obviously influenced by the prevailing microbiota, but in turn will evidently influence the sourdough microbiota by initiating an acidic stress through the drop of the pH during (natural) acidification upon sourdough fermentation (Gänzle and Gobbetti, 2013; Guerzoni et al., 2013). For instance, yeasts are particularly sensitive to undissociated acetic acid. The production of lactic acid, and to a lesser extent, acetic acid by LAB is the main driving force behind the pH drop in sourdough and hence provides a selective environment for the LAB communities. Many sourdough-adapted LAB are therefore characterized by a high acid tolerance, although the influence of the pH on the microbial ecology also depends on the duration of the fermentation time between the backslopping steps (De Vuyst et al., 2014; Gobbetti et al., 2005). Highly acidified sourdoughs are dominated by, among others, *Lb. plantarum*, *Lb. fermentum*, *Lactobacillus reuteri*, or *Lactobacillus rossiae* (Van der Meulen et al., 2007; Vogelmann and Hertel, 2011; Vrancken et al., 2011b, Weckx et al., 2010b). Oppositely, the sourdough-specific *Lactobacillus sanfranciscensis* does not survive below pH 3.8 and is therefore not found in highly acidic sourdoughs, but is often encountered in mildly fermented wheat sourdoughs with a final pH of 4.0 or higher (De Vuyst et al., 2014; Gänzle et al., 1998). Yet, *Lb. sanfranciscensis* does survive sourdoughs with low pH upon frequent backslopping; similarly, acid tolerance of *Lb. reuteri* does not contribute to its competitiveness in sourdoughs with short backslopping times (De Vuyst et al., 2014; Gänzle and Gobbetti, 2013). In addition, species of the genera *Leuconostoc*, *Pediococcus*, and *Weissella* often dominate sourdoughs with a pH above 4.0 (De Vuyst et al., 2014; Minervini et al., 2012; Robert et al., 2009). It is however unclear whether the prevalence of these species is due to the pH above 4.0 of the mature sourdoughs and/or whether this is due to other factors, such as the dough yield or fermentation time that select for these microbial communities.

This paper aimed at unraveling whether the pH of the mature sourdough is a consequence of the prevailing microbiota, in particular the LAB communities, or whether the pH determines the LAB and yeast community dynamics. Therefore, the influence of a pH kept constant at the crucial value of 4.0 on spontaneously developed backslopped liquid wheat sourdoughs was examined in detail.

2. Materials and methods

2.1. Flour

All sourdoughs were prepared using white wheat flour with the following specifications: humidity, 14.0–14.5%; protein content on dry matter, 12.7–12.9%; ash content on dry matter, 0.59–0.60%; and Hagberg falling number, 307–285 s.

2.2. Set-up of the fermentations

Spontaneously initiated liquid sourdough fermentations (8 kg) were carried out in 15-L Biostat C fermentors (Sartorius, Melsungen, Germany), filled with 6 L of sterile water and 2 kg of wheat flour, with a resulting dough yield [(dough mass / flour mass) × 100] of 400. The first fermentation was carried out at 30 °C for 24 h, while the mixture was kept homogeneous through stirring (300 rpm). The headspace of the fermentors was flushed with sterile air at a flow rate of 1 L/min to ensure a stable pressure and chemical composition of the headspace (necessary for the online measurements, see Section 2.7.1). After the initial fermentation, 800 mL of sourdough was harvested and used as an inoculum for a subsequent backslopped sourdough fermentation, consisting of 5.4 L of sterile water and 1.8 kg of wheat flour, adding up to a total volume of 8 kg. This backslopping procedure was continued for ten days. Two such experiments were set up. Sourdough fermentation experiment I consisted of ten backslopping steps with free-pH evolution after each refreshment, further referred to as backslopping steps I-1 to I-10. After nine backslopping steps, the sourdough of backslopping step I-9 was also used for a subsequent backslopping step (I-11), followed by a final backslopping step (I-12), both with the pH kept constant at a minimum of 4.0 through automatic addition of a 5 M NaOH solution, as to assess the adaptation of the microbial communities to the changed pH conditions. After 24 h of fermentation, backslopping steps I-11 and I-12 were continued for a prolonged fermentation. Likewise, sourdough fermentation experiment II consisted of ten backslopping steps with the pH kept constant at a minimum of 4.0, further referred to as backslopping steps II-1 to II-10. The sourdough of backslopping step II-9 was also used for a subsequent backslopping step (II-11), followed by a final backslopping step (II-12), both with free-pH evolution, as to assess the capacity of the microbial communities developed to acidify the sourdough below pH 4.0.

2.3. Sampling

Upon the backslopping procedure, sourdough samples were withdrawn prior to each backslopping step as well as at regular time intervals during fermentation after refreshment. Additional samples were taken after 48 and 96 h during prolonged fermentation of backslopping steps I-11 and I-12. A flour sample was taken too. Each time, two samples of 50 g of ripe sourdough were taken to analyse the physicochemical characteristics/microbial community dynamics and substrate consumption/metabolite production kinetics, respectively. The freshly taken samples were centrifuged (6201 ×*g* for 30 min at 4 °C) to remove flour particles and bacterial cells, after which the sediment was discarded, and the cell-free supernatants were transferred into 2-mL vials (Sarstedt, Nümbrecht, Germany). For culture-independent microbiological analysis, 10 g of freshly taken sourdough sample were mixed with 40 g of maximal recovery agent [NaCl (Merck, Darmstadt, Germany), 0.85%, m/v; peptone (Oxoid, Basingstoke, Hampshire, United Kingdom), 0.1%, m/v], agitated for 30 min at 4 °C, and centrifuged at 120 ×*g* for 10 min at 4 °C. The solids were discarded and the supernatants were centrifuged at 6201 ×*g* for 30 min at 4 °C to collect the cells. The cell pellets obtained were stored at −20 °C until further analysis. For GC–MS sampling, 5 g of fresh sourdough sample were mixed with 1 g of NaCl in 20-mL glass headspace vials (Gerstel, Mülheim-an-der-Ruhr, Germany), which were closed with a magnetic screw cap with polytetrafluoroethylene/silicone septum (Gerstel). All samples were frozen at −20 °C and thawed immediately prior to analysis.