Available online at www.sciencedirect.com
SCIENCE DIRECT®
LWT 39 (2006) 256–265
LWT
www.elsevier.com/locate/lwt

Study of the behaviour of *Lactobacillus plantarum* and *Leuconostoc* starters during a complete wheat sourdough breadmaking process

Hervé Robert, Valérie Gabriel, Dominique Lefebvre, Philippe Rabier, Yves Vayssier, Catherine Fontagné-Faucher*

Laboratoire de Biologie Appliquée à l'Agroalimentaire et à l'Environnement (LBAE), Département Génie Biologique, IUT Paul Sabatier, 24 rue d'Embaquès 32000 Auch, France

Received 5 August 2004; accepted 21 January 2005

Abstract

The acidification properties, metabolic activity and technological performance of four individual *Lactobacillus plantarum* or *Leuconostoc* freeze-dried starters were investigated during a complete wheat sourdough breadmaking process including 0.2 g/100 g baker's yeast. Microbiological contents (lactic acid bacteria and yeasts), acidification characteristics (pH and total titratable acidity), soluble carbohydrates (maltose, glucose and fructose) and fermentative end-products (lactic and acetic acids, ethanol) contents were evaluated during both sourdough and corresponding bread dough fermentation. Biochemical and technological analysis of the resulting bread products are also presented. Some differences among strains in acidification properties and soluble carbohydrates availability were outlined both in sourdough and bread dough. Each individual *Leuconostoc* or *Lb. plantarum* starter was able to produce a characteristic fermentation and was found to ensure the production of breads with overall satisfactory acceptance.

Keywords: Sourdough; Fermentative end-products; Flour soluble carbohydrates; *Lactobacillus plantarum*; *Leuconostoc* sp.

1. Introduction

The use of sourdough in wheat bread production clearly improves dough properties, bread texture and flavour, delays the staling process and prevents bread from mould and bacterial spoilage (Gobbetti, 1998; Hammes & Gänzle, 1998; Martinez-Anaya, 2003). These benefits result from an appropriate balance between the metabolism of yeast and hetero- and homo-fermentative lactic acid bacteria (LAB) strains that represent the predominant microorganisms in natural sourdoughs. LAB metabolism is responsible for the production of organic acids and contributes, along with yeasts, to the production of aromatic compounds (Damiani et al., 1996; Martinez-Anaya, 1996a; Meignen et al., 2001). Sourdough microflora generally contain a complex mixture of yeasts (mainly *Saccharomyces cerevisiae*) and hetero- and homo-fermentative LAB. A large number of *Lactobacillus* species have been isolated including *Lactobacillus sanfranciscensis*, *Lb. pontis, Lb. brevis* and *Lb. plantarum* strains the most frequently described (Ottogali, Galli, & Foschino, 1996; Gobbetti, 1998; De Vuyst et al., 2002). Despite a predominance of lactobacilli, LAB cocci, belonging to *Leuconostoc* and *Pediococcus* genera, were also identified in traditional wheat sourdough from European countries (Infantes & Tourneur, 1991; Gabriel, Lefebvre, Vayssier, & Faucher, 1999; Corsetti et al., 2001). Starters composed of specific individual LAB, or mixed LAB and yeasts, became available a few years ago allowing the production of a full sourdough in a one-stage process. Such commercial starters improve the control of the sourdough production while ensuring reliable quality in bread production. The design of starters requires prior

*Corresponding author. Tel.: +33 5 6261 6312; fax: +33 5 6261 6301.
E-mail address: cathy.faucher@iut-tlse3.fr (C. Fontagné-Faucher).

0023-6438/$30.00
doi:10.1016/j.lwt.2005.01.013

knowledge of the biochemical characteristics and baking potential of the microorganisms. Performance of LAB isolates has mainly been studied by characterization of the acidification properties such as pH, total titratable acidity (TTA) and lactic and acetic acids production during sourdough fermentation (Collar, 1996; Corsetti et al., 1998; Hammes & Gänzle, 1998). Furthermore, acetate production by heterofermentative metabolism is of major importance for the development of flavour. The molar ratio between lactic to acetic acid in bread (fermentation quotient, FQ) is considered optimum in the range between 2.0 and 2.7 (Hammes & Gänzle, 1998). Production of suitable end-products during dough fermentation depends on the availability of soluble carbohydrates. In wheat flour, the total concentration of maltose, sucrose, glucose and fructose is rather low and varies from 1.5 to 2 g/100 g, maltose being the most abundant fermentable carbohydrate. Important changes in carbohydrate fractions occurred during sourdough fermentation resulting from both enzymatic activities of the flour and metabolic conversions by microbial enzymes (Collar, 1996; Martinez-Anaya, 1996b; Gobbetti, 1998). Metabolism of carbohydrates varies depending on the LAB species, and even strains, the type of sugars, the co-presence of yeasts and the processing conditions (Rouzaud & Martinez-Anaya, 1993; Gobbetti, Corsetti, & Rossi, 1994; Martinez-Anaya & Rouzaud, 1996). The soluble carbohydrates remaining after microbial fermentation participate in browning reactions during baking, contributing to organoleptic characteristics of the bread (Collar, 1996). Furthermore, in most industrial applications, a variable amount of baker's yeast is added to bread doughs as leavening agent together with the sourdough preparation (Hammes & Gänzle, 1998). Therefore, baker's yeast enzymes may interfere with the metabolic activities of the sourdough microflora during the breadmaking process.

Organic acid production and carbohydrate metabolism during sourdough fermentation depend to various extents on microbial starter composition and on process parameters such as flour ash content, dough yield, fermentation time and temperature and NaCl concentration (Martinez-Anaya, Pitarch, Bayarri, & Benedito de Barber, 1989; Martinez-Anaya, Grana, & Torner, 1993; Martinez-Anaya, Benedito de Barber, & Collar Esteve, 1994; Gobbetti et al., 1995; Gianotti et al., 1997; Rouzaud & Martinez-Anaya, 1997; Meignen et al., 2001). All of the above studies have focused on the metabolic activities of individual isolates or mixed starters using species such as *Lb. sanfranciscensis*, *Lb. plantarum*, and *Lb. brevis*. In addition, a previous study in our laboratory on the characterization of the LAB microflora of traditional wheat sourdoughs from the Midi-Pyrénées region (France) has revealed the importance of *Leuconostoc* genera since such heterofermentative cocci were detected associated with *Lb. plantarum* in all the nine sourdoughs tested (Gabriel et al., 1999). As far as we know, no other study which included *Leuconostoc* strains has been reported concerning wheat bread fermentation. The acidification potential of *Leuconostoc mesenteroides* strains was only checked during rye sourdough fermentation (Lönner & Preve-Akesson, 1988) and pizza dough fermentation (Coppola, Pepe, & Mauriello, 1998).

The aim of this study was to evaluate the behaviour of four individual freeze-dried starters including *Lb. plantarum* (homofermentative), *Leuconostoc citreum* and *Leuc. mesenteroides* (heterofermentative) during a complete wheat sourdough breadmaking process including baker's yeast addition. Microbiological, biochemical, physico-chemical and technological analyses have been performed on sourdoughs, and on bread doughs over the fermentative period and on final bread products.

2. Materials and methods

2.1. Microorganisms

Four LAB strains, previously isolated from traditional French wheat sourdoughs, were used in this study: *Lactobacillus plantarum* AELLI12 and EMRS4, *Leuconostoc citreum* BELLI7 and *Leuc. mesenteroides cremoris* AMSE2 (Gabriel et al., 1999). These strains have been found to be able to ferment maltose, glucose and fructose and were identified by 16S ribosomal DNA (rDNA) sequencing (Molecular typing centre, Institut Pasteur, Lille, France). These strains were used in a lyophilized form with approximately 1×10^{10} cfu/g (Bioprox, France). Commercial compressed baker's yeast (*Saccharomyces cerevisiae*) was used during the breadmaking process (Lesaffre, Marcq-en-Baroeul, France).

2.2. Sourdough preparation and fermentation

Wheat flour for traditional breadmaking (T65 as according to the French classification on ash content, malt flour <0.1 g/100 g, without ascorbic acid) was used (Gers Farine Minoterie, France). The main characteristics of the flour were: moisture 15.6 g/100 g, protein 11.5 g/100 g, damage starch fraction 7.5 g/100 g and falling number 275 s.

Sourdough preparation in laboratory conditions included 250 g wheat flour, 150 ml tap water, 3.8 g salt and 10 ml starter suspension in order to produce a firm dough with an initial viable counts of about 1×10^7 LAB cfu/g (dough yield DY = 160). After kneading with continuous speed mixer (60*g*) for 10 min at room temperature, dough samples were divided into 10 g