LACTIC ACID BACTERIA IN RYE SOURDOUGH FROM CRUDE AND PEELED RYE FLOUR

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Abstract

In Latvia the spontaneous sourdough is used in traditional rye bread baking whose microflora is determined in flour and in microorganism cultures presented in external environment. Almost all spontaneous sourdough cultures, especially those that have been maintained for a long time, contain both lactic acid bacteria (LAB) and yeasts. The main purpose of the current research was to analyze growth dynamics of LAB in spontaneous rye flour sourdough and to isolate some of its representatives. Experiments were carried out in Department of Food Technology, Faculty of Food Technology, Latvia University of Agriculture in January and February 2008. Considering differences in constituents, two types of flour were used in the research – peeled and crude rye flour. There were three stages of spontaneous sourdough preparation in 72 hours totally; the renewal of sourdough was realized each 24 hours. The dynamics of LAB plate count in every stage of fermentation was investigated as well as changes of pH was observed using standard methods. The results of experiments show substantial increase in amount of LAB in both sourdoughs, particularly in sourdough from peeled flour, reaching 6.06 log10 cfu ml^{-1}. A significant decrease of pH value from pH 6.7 to pH 3.8 during fermentation process was observed. As a result, the sourdough from peeled flour had desirable properties for preparation of sourdough starter. LAB cultures isolated and identified from current sourdoughs using API tests: *Lactobacillus brevis* and *Lactobacillus fermentum* are also typical members of sourdoughs found in other countries.

Key words: lactic acid bacteria, sourdough, rye flour.

Introduction

Sourdough is essential in rye bread making and the tradition of rye sourdough fermentation correspond to the rye-growing areas in north, central and eastern European countries including the Baltic states, where rye bread constitutes a considerable amount of the bread consumption (Rocken, 1996). Traditional sourdough bread technology is based on a spontaneous fermentation process from Lactic acid bacteria (LAB) and yeast occurring naturally in flour. Classic sourdough preparation is a multiple stage process that starts with a mixture of flour and water left for a specific period of time. Every next stage is prepared with fresh flour and water added to the previous stage (Linko et al., 1997; Kariluoto et al., 2004). In the first stage of sourdough fermentation the temperature vary from 25 °C to 26 °C, which is optimal for yeast development. In the second and third stage of sourdough fermentation an average temperature of 32 °C is applied – optimal for lactic acid bacteria (Kramer, 2002).

The character of the process results from the growth of microorganisms in different environmental conditions. Temperature, dough consistence and dough resting time at each stage determine development of active microflora (Javanainen and Linko, 1993; Muller et al., 2001).

In addition to environmental influence, flour is largely responsible for the properties and quality of spontaneously fermented sourdough. Crude rye flour contains more cereal outer layers resulting in greater diversity of microorganisms inhibiting growth of LAB comparing with peeled rye flour. Rye flour naturally contain a wide variety of yeasts and bacteria – *Candida crusei, Erwinia herbicala, Bacillus spp.*, moulds, *Saccharomyces spp.,* heterofermentative LAB and acid – tolerant yeasts (Kramer, 2002).

Sourdough fermentation begins with aerobic growth immediately upon mixing flour and water. Once oxygen is depleted, anaerobic fermentation begins with the growth of LAB, which produces acids that enhance their rapid growth when the pH value has dropped too low for other microorganisms to develop. Thereby LAB becomes the most abundant microorganisms in the sourdough and they are therefore responsible for the final stages of the sourdough processing (Savič et al., 2006).

Genera of LAB identified from sourdoughs

are *Lactobacillus, Leuconostoc, Pediococcus* and *Streptococcus*, and the majority of the sourdough LAB belongs to the genus *Lactobacillus*. The taxonomy of LAB is still under revision. *Lactobacillus* present in sourdough has been divided in three groups according to their carbohydrate fermentation patterns:

- Obligately homofermentative LAB: *L. acidophilus, L. delbrueckii ssp. bulgaricus, L. farciminis* etc. Hexoses are almost completely fermented to lactic acid (>85%) by the Embden-Meyerhof-Parnas (EMP) pathway. Fructose is also fermented, but neither gluconate nor pentoses are fermented.
- Facultatively heterofermentative LAB: *L. casei, L. curvatus, L. plantarum* etc.

Hexoses are almost completely fermented to lactic acid by the EMP pathway. Pentoses are fermented to lactic acid and acetic acid by an inducable phosphoketolase.

- Obligately heterofermentative LAB: *L. brevis, L. fermentum, L. fructivorans* etc.

Hexoses are fermented to lactic acid, acetic acid, ethanol, and CO_2. Pentoses are fermented to lactic acid and acetic acid. In general, both pathways involve phosphoketolase (Kandler and Weiss, 1986).

Scientific publications show that application of spontaneous sourdough in rye bread production may cause unstable quality of rye bread (Reed and Nagodawithana, 1995). Selected LAB starter cultures should be used in Latvian bakeries to provide controlled sourdough fermentation. Though LAB starters selected in Europe frequently does not satisfy Latvian bakers. Therefore, the aim of the research was to analyze growth dynamics of LAB in spontaneous sourdough from peeled and crude rye flour at every fermentation stage and to isolate some of its representatives.

When the above mentionedis clarified, it is possible to promote viability and development of LAB providing the highest acidity and preferable sensory properties accommodating technological processes of sourdough fermentation – length of every stage, temperature of fermentation and flour – water proportion in favour of it.

The aim of the current research was to analyze growth dynamics of LAB in spontaneous rye flour sourdough and to isolate some of its representatives.

Material and Methods

Current research was carried out in Latvia University of Agriculture in the Department of Food Technology in Scientific Laboratory of Microbiology in 2008.

The rye flour from stock company 'Jelgavas dzirnavas': peeled rye flour (ash content 1.45%, moisture content 14.5%), crude rye flour (ash content 1.85%, moisture content 14.5%) and water were used in all samples. There were three stages of sourdough preparation totally 72 hours; the renewal of sourdough was realized each 24 hours (Figure 1).

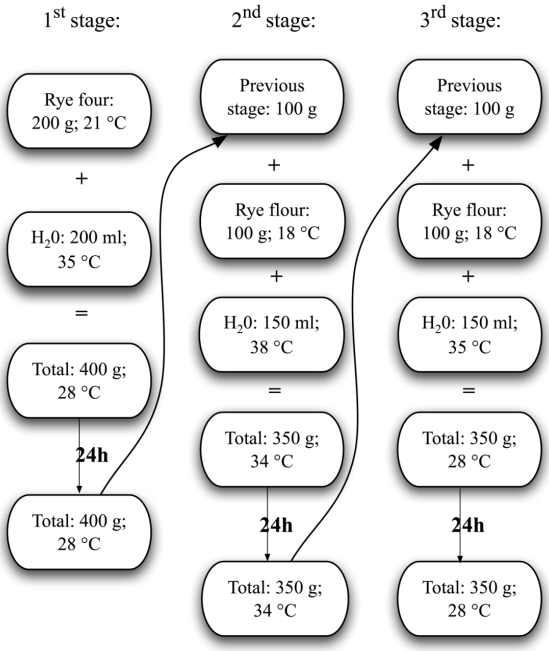

Figure 1. Three-stage technological process of spontaneous rye flour sourdough preparation.

Ten grams of sourdough in 90 ml 0.5% sterile physiological liquid were mixed in BagMixer® at speed 7 for 60 seconds after each stage of sourdough preparation.

Plate counting method was used for microbial detection. The samples for investigation were taken in: 0, 4th, 8th, 24th, 28th, 32nd, 48th, 52nd, 56th, 72nd hour of fermentation.

Lactic acid bacteria plate count was investigated on MRS agar (dilutions 1:100; 1:1000) in two replications. Incubation was performed at 35 °C for 24 hours to develop the colonies.

Counting of colonies formed and calculating the number of CFUs was accomplished by Acolyte colony counter.

Changes of pH (Jenway 3250 pH meter) in sourdough were observed using standard methods with reference to 'Standard - Methoden fur Getreide, Mehl und Brot' (Spicher and Stephen, 1993).

Dilution and Lindner methods were applied to obtain pure cultures. After isolating pure LAB cultures API CH 50 identification method was applied to identify microorganism species.

A standard method of arithmetic mean was used in data processing.

Results and Discussion

Three-stage method was used in spontaneous rye sourdough preparation (Figure 1). At the end of each stage, dynamics of LAB development was investigated.

Results shown in Figure 2 characterize growth dynamics of LAB in spontaneous sourdough fermentation process using two types of flour – peeled and crude rye flour. Initial rate of plate count was 3.48 log10 cfu ml^{-1} for crude flour sourdough and 4.27 log10 cfu ml^{-1} for peeled flour sourdough. It could be explicable with variety of microorganisms inhibiting growth of LAB in crude rye flour (Kramer, 2002). In the first stage of fermentation the development of LAB was significant in crude flour sample – an amount of LAB increased by 54% reaching 5.37 log10 cfu ml^{-1} exceeding plate count of LAB in peeled flour sourdough by 13%. After the first renewal of sourdough LAB count in crude flour sourdough decreased by 8% and remained stable at the level of 5 log10 cfu ml^{-1} until the middle of the third stage of fermentation. LAB plate count in peeled flour sourdough increased gradually reaching 6.02 log10 cfu ml^{-1} at the end of second stage of fermentation and remained stable until the end of fermentation process. The second renewal at the beginning of the third stage of fermentation had insignificant influence on development of LAB in both sourdoughs. It is possible that LAB cells were ageing and metabolites present in dough were inhibiting its regeneration. Finally, the amount of LAB in peeled flour sample exceeded the amount of LAB in crude flour sample by 13%, evidencing the advantage of using peeled rye flour instead of crude rye flour as a starter.

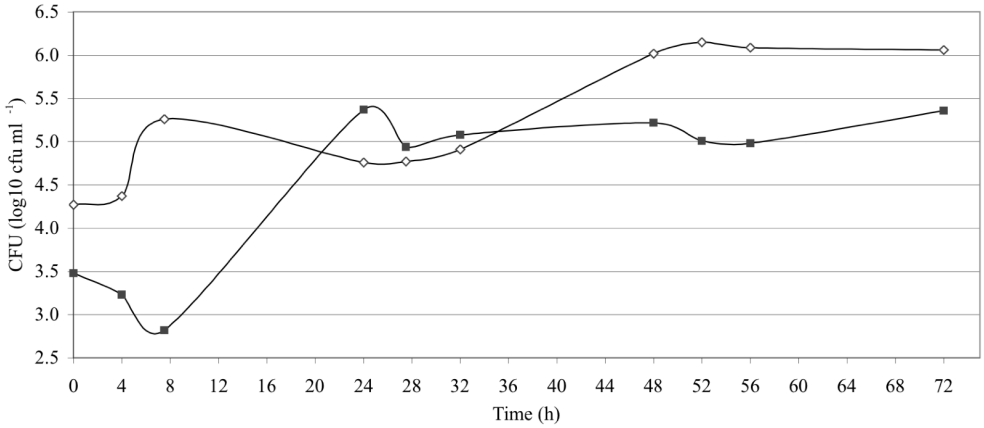

Figure 2. Development of LAB in spontaneously prepared sourdough from peeled and crude rye flour during 72 hours (◊ - sourdough from peeled rye flour; □ - sourdough from crude rye flour)

Figure 3 represent development of LAB and changes of pH value in peeled flour sample during three- stage fermentation process. At the first four hours of fermentation changes in total amount of LAB and pH value were not relevant – microorganisms remained in lag – phase and adapted to the new nutrients available. After four hours LAB started an intensive exponential phase although at the

end of the first stage of sourdough fermentation LAB plate count started to decrease caused by limitation of nutrients. Generally, in the first stage of fermentation pH value decreased substantially as a result of intensive development of microorganisms – from initial rate of pH 6.7 to pH 4.26.

Immediately after the first renewal of sourdough pH value increased rapidly to pH 5.37 but after four hours it returned close to a previous level to pH 4.36. At the same time LAB started a new lag-phase. At the end of the second stage of spontaneous sourdough preparation, LAB were developed rapidly in exponential growth phase by 26% and reached 6.02 log10 cfu ml^{-1}. In the third stage of fermentation an amount of LAB cells remained relatively high and stable. Furthermore, the pH value continued to decrease until the end of the third stage reaching pH 3.83 representing that current sourdough has desirable properties for preparation of rye flour sourdough starter.

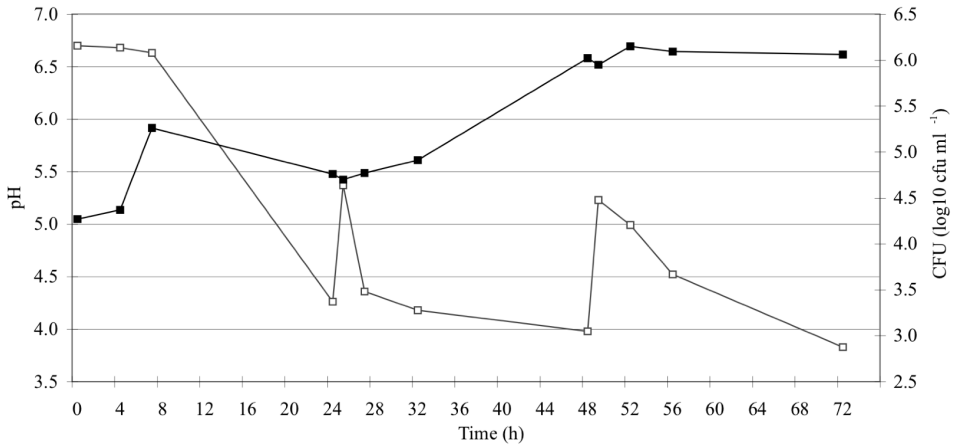

Figure 3. Development of LAB and changes of pH value in spontaneously prepared sourdough from peeled rye flour during 72 hours (□ - pH; ■ - CFU)

Figure 4 represents development of LAB and changes of pH value in crude flour sourdough. Analogical to peeled flour sourdough, pH changes in the first hours of fermentation were not relevant, but the amount of LAB cells even decreased by 19%. After eight hours of fermentation, LAB developed rapidly in exponential growth phase by 90% and reached 5.37 log10 cfu ml^{-1} at the end of fist stage of fermentation. Considering vast changes in growing media, the first renewal of sourdough inhibited growth of LAB by 8% although at the end of the second stage of fermentation, LAB cell count continued to increase gradually. During the second stage of fermentation value of pH decreased substantially by 38% reaching pH 3.81 as a result of metabolites produced by LAB in stationary phase. Despite the final pH value 3.80, during the third stage of fermentation LAB continued to develop reaching 5.36 log10 cfu ml^{-1} which is not sufficient for preparation of rye flour sourdough starter.

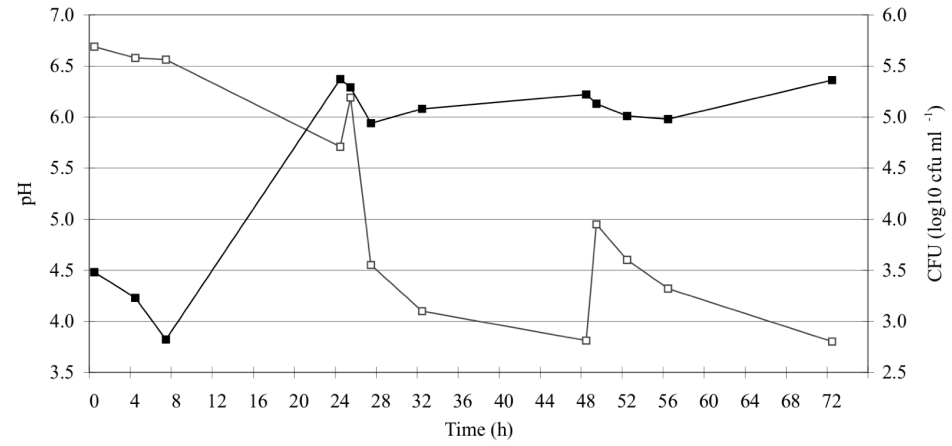

Figure 4. Development of LAB and changes of pH value in spontaneously prepared sourdough from crude rye flour during 72 hours (□ - pH; ■ - CFU)

Results of API tests reveal LAB that are typical members of rye flour sourdough microflora - *Lactobacillus brevis* and *Lactobacillus fermentum*. With reference to 'Bergey's Manual of Systematic Bacteriology' (Kandler and Weiss, 1986): *L. brevis* and *L. fermentum* belong to heterofermentative LAB, producing about 50% of end products from glucose as lactic acid, with considerable amounts of CO_2, acetic acid and ethanol; mannitol from fructose; *L. fermentum* are able to grow at 45 °C, but not at 15 °C; *L. brevis* are able to grow at 15 °C, but not at 45 °C. The latter properties ensure activity of LAB in every stage of spontaneous sourdough preparation. With reference to 'Handbook of food science, technology and engineering' (Hui, 2006): heterofermentative LAB *Lactobacillus brevis* are found in rye bread sourdough from Russia, Germany and Sweden; *Lactobacillus fermentum* are found in German, Austrian and Swedish rye bread sourdoughs.

Conclusions

1. During 72 hours of rye sourdough preparation process, the amount of LAB increased by 42% in sourdough from peeled flour and by 54% in sourdough from crude flour, though activity of LAB increased significantly considering pH value changes from pH 6.7 to pH 3.83 (peeled rye flour) and from pH 6.69 to pH 3.80 (crude rye flour).

2. High amount of LAB reaching 6.06 log10 cfu ml^{-1} and a final pH value 3.83 represent that sourdough from peeled rye flour has desirable properties for preparation of sourdough starter.

3. After 72 hours of fermentation, spontaneous sourdough from peeled rye flour contains 13% more LAB than sourdough from crude rye flour. This interconnection is stable during fermentation process.

4. LAB cultures isolated and identified from current sourdoughs: *Lactobacillus brevis* and *Lactobacillus fermentum* are also typical members of German, Russian, Swedish etc. traditional rye flour sourdough.

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