CHARACTERIZATION OF RYE SOURDOUGH MICROFLORA

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Abstract

Preparing of sourdough is one of the oldest biotechnological methods, but the research is still going on and is crucial. In Latvia the spontaneous sourdough is used in traditional rye bread baking which microflora is determined in flour and in microorganisms cultures presented in external environment. Literature data proved that spontaneous sourdough presents several lactic acid bacteria (LAB) and yeasts. Lactobacilli present in sourdough are both homofermentative and heterofermentative and depending on temperature can be presented as well as mesophylic and thermophilic. The latter present in scalding are essential to ensure technologocal process of rye bread. Metabolites of thermophilic LAB are responsible for providing sufficient dough acidity. Besides LAB sourdough contain yeasts including *Saccharomyces cerevisiae, Pichia saitoi, Candida crusei, etc*. The aim of the research was to analyze growth dynamics of microflora in three-stage spontaneous rye flour sourdough fermentation process and to isolate some of its representatives. One of the basic tasks for applying the research was to acquire methods of micro-organism identification. Results of experiments show predominance of LAB reaching 6.06 log10 cfu ml^{-1}, high amount of yeasts reaching 5.22 log10 cfu $ml^{-1}$and a final pH value 3.83 representing that this sourdough has desirable properties for preparation of rye flour sourdough starter. For identification of LAB cultures API CH 50 test was acquired while ID 32 C for yeasts. Results of the experiments reveal heterofermentative LAB *Lactobacillus Brevis* and *Saccharomyces cerevisiae* yeast presence in sourdough. These microorganisms are typical members of sourdough microflora with reference to foreign scientific publications.

Key words: sourdough microflora, lactic acid bacteria, yeasts

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 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 form 25 ℃ to 26 ℃, which is optimal for yeast development. In the second and third stage of sourdough fermentation an average temperature of 32 ℃ is applied – optimal for lactic acid bacteria (Kramer, 2002).

The character of the process results from 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. Rye flour naturally contains a wide variety of yeast and bacteria – *Candida crusei, Erwinia herbicala, Bacillus spp.*, moulds, *Saccharomyces spp.,* heterofermentative LAB and acid – tolerant yeasts (Kramer, 2002).

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: Obligate homofermentative LAB - *L. acidophilus, L. delbrueckii* spp. *bulgaricus, L. farciminis* etc.; Facultatively heterofermentative LAB: *L. casei, L. curvatus, L. plantarum* etc.; Obligately heterofermentative LAB: *L. brevis, L. fermentum, L. fructivorans etc*. (Kandler and Weiss, 1986).

The most frequently isolated yeast species from rye and wheat sourdoughs are *Saccharomyces cerevisiae* which are able to ferment glucose, galactose, maltose and raffinose, but not lactose.

Other yeast species often isolated from sourdoughs are *S. exiguus, Candida milleri (C. holmii), C. krusei*. The latter are able to ferment glucose only. The yeast species *Pichia sitoi, P. norvegensis* and *Hansenula anomala* and some *Saccharomyces* spp. have occasionally been isolated from sourdoughs (Manyenm *et al.,* 1999).
Further the LAB of the sourdough have a synergistic effect with yeasts and inhibit the growth of molds and of rope (*Bacillus mesentericus*) (Reed and Nagodawithana, 1995).
There are relatively few investigations regarding LAB and yeast interaction in sourdough. *Lactobacillus spp.* is the main producer of organic acids in spontaneously fermented sourdough. Although the decrease of pH may cause metabolites (organic acids, ethanol, etc) of *Enterobactericae,* moulds or acid – tolerant yeasts present in spontaneous sourdough (Kramer, 2002).
Bacillus spp. represents undesirable microflora in spontaneous sourdough. For example if *Bacillus subtilis* has expanded in dough, it produces proteolases and hydrolyse proteins resulting in increasing of dough pH and preventing of LAB and yeast activity. Besides the metabolites of *Bacillus subtilis* generate undesirable taste and aroma of dough (Kramer, 2002).
Scientific publications show that application of spontaneous sourdough in rye bread production may cause unstable quality of rye bread. 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 dynamics of spontaneous rye flour sourdough microflora development in every fermentation stage was investigated and some of its representatives were isolated.
When the above is clarified it is possible to promote viability and development of desirable microflora accommodating technological processes – length of every stage, temperature of fermentation and flour – water proportion in favour of it. Desirable microflora in this case refers to LAB and yeasts providing the highest acidity and preferable sensory properties.

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" (ash content 1.45%, moisture content 14.5%) and water was 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).
10 grams of sourdough in 90ml 0.5% sterile physiological liquid were mixed in BagMixer® at speed 7 for 60 seconds.
Plate counting method was used for microbial detection. The samples for investigation in two reiterations were taken in: 0, 4, 8, 24, 28, 32, 48, 52, 56, 72 hour of fermentation.
Total plate count was investigated on Nutrition agar (dilutions 1:100; 1:1000; 1:10000). Yeast plate count was investigated on Malt extract agar (dilutions 1:100; 1:1000). Lactic acid bacteria plate count was investigated on MRS agar (dilutions 1:100; 1:1000). Incubation was performed at 35 °C (for total plate count and LAB) and 27 °C (for yeasts) 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 für Getreide, Mehl und Brot" (Spicher, 1993).

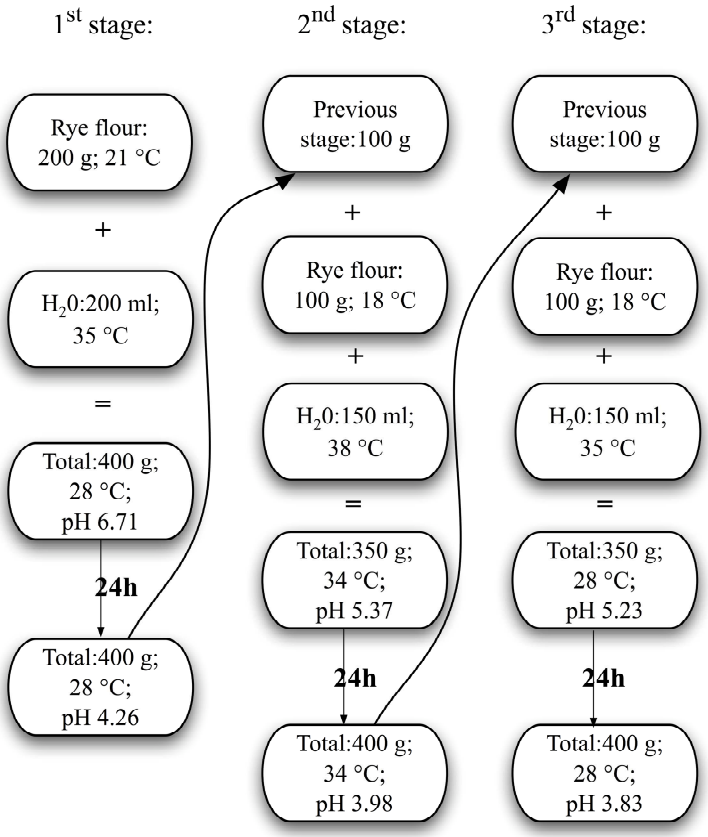

Figure 1. Three-stage technological process of spontaneous rye flour sourdough preparation

Dilution and Lindner methods were applied to obtain pure cultures. After isolating pure cultures API identification method was applied to identify microorganism cultures. For identification of LAB cultures API CH 50 test was used while ID 32 C for yeasts.

Results and Discussion

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

Results shown in Figure 2 represent growth dynamics of spontaneous sourdough microflora during fermentation process and changes of pH value. The initial rates of plate count were very close – 4.27 log10 cfu ml^{-1} (LAB), 4.54 log10 cfu ml^{-1} (yeasts), 4.72 log10 cfu ml^{-1} (total plate count).

At the first four hours of fermentation changes in total amount of microorganisms and pH value were not relevant – LAB and yeasts remained in lag – phase and adapted to the new nutrients available. After four hours LAB and yeasts started an intensive exponential phase although at the end of the first stage of sourdough fermentation LAB and particularly yeast plate count started to decrease caused by limitation of nutrients.

Amount of yeast cells became 28% lower than initial rate supposedly because of activity of LAB. Generally, in the first stage of fermentation pH value decreased substantially as a result of intensive development of microorganisms – from initial rate 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 whereas amount of yeasts increased by 31% in four hours after renewal. 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}. Equally, growth of yeasts increased in exponential growth phase during the second stage of fermentation. Decrease of pH value was not significant but remained stable and reached pH 3.98.

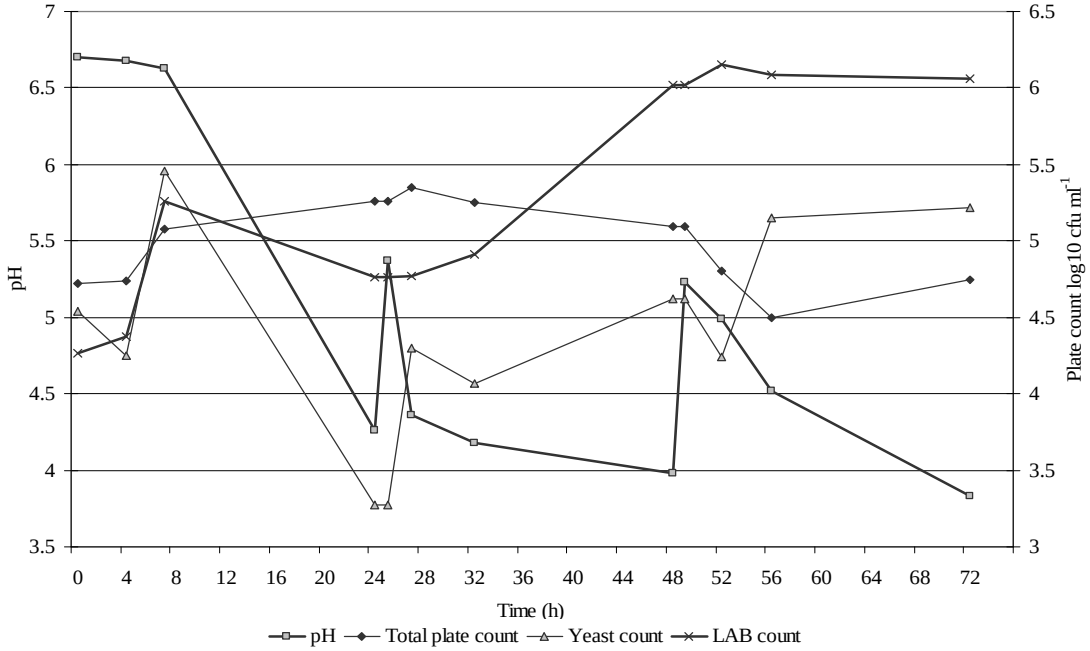

Figure 2. Development of microorganisms and changes of pH value in spontaneously prepared sourdough from rye flour during 72 hours

The second renewal at the beginning of third stage of fermentation had insignificant influence on development of LAB. It is possible that LAB cells were ageing and metabolites present in dough were inhibiting its regeneration. Opposite results were observed in the changes of yeast content – it increased by 23% during the third stage of fermentation and reached 5.22 log10 cfu ml^{-1}.

Convincing predominance of LAB, high amount of yeasts and a final pH value 3.83 represent that current sourdough has desirable properties for preparation of rye flour sourdough starter.

Results of API tests reveals microorganisms that are typical members of rye flour sourdough microflora - *Lactobacillus brevis, Lactobacillus fermentum, Saccharomyces cerevisiae.* With reference to "Handbook of food science, technology and engineering" (Y. H. 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 sourdough; *Saccharomyces cerevisiae* yeast present microflora of rye flour sourdough from Germany, Finland, Poland, Germany and Denmark.

Conclusions

1. During 72 hours of rye flour sourdough preparation process, amount of LAB and yeasts increased by 42% and 15% respectively, though activity of these microorganisms increased significantly considering pH value changes from pH 6.7 to pH 3.83.
2. Predominance of LAB reaching 6.06 log10 cfu ml^{-1}, high amount of yeasts reaching 5.22 log10 cfu ml^{-1}and a final pH value 3.83 represent that this sourdough has desirable properties for preparation of rye flour sourdough starter.
3. Identification of microorganisms using API test method was successfully acquired. LAB and yeast cultures isolated and identified from current sourdough: *Lactobacillus brevis, Lactobacillus fermentum, Saccharomyces cerevisiae* are also typical members of German, Russian, Swedish etc. traditional rye flour sourdough.

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