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review

Application of Starter-cultures for Baked Goods

Primjena mikrobnog cjepiva u pekarstvu

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Summary

Nowadays the baker's yeast of high activity and stability is produced in all developed countries. It satisfies modern procedures of fast preparation and short-time fermentation of dough in order to speed up the bread production time. However, this production does not completely meet the quality demands for bread and other bakery products.

Many researchers have investigated the influence of different microorganisms added to common baker's yeast for sour dough fermentation and final products quality, in an attempt to achieve a better quality of bakery products.

The aim of the present paper is to clarify the role of lactic acid bacteria in pure and mixed cultures with yeasts used in baked goods production.

Sažetak

Danas se u svim razvijenim zemljama proizvodi visokoaktivan i stabilan pekarski kvasac koji zadovoljava suvremene postupke brzog zamjesa i kratke fermentacije kako bi proizvodnja kruha što kraće trajala. Međutim, takvom proizvodnjom nije u potpunosti zadovoljena kakvoća kruha i ostalih pekarskih proizvoda.

Radi što bolje kakvoće pekarskih proizvoda, mnogi su istraživači ispitivali utjecaj različitih mikroorganizama dodanih standardnom pekarskom kvascu za fermentaciju kiselog tijesta.

Zato je svrha ovog prikaza objašnjenje uloge bakterija mliječno-kiselog vrenja u čistim i mješovitim kulturama s kvascima koji se primjenjuju u pekarstvu.

Introduction

Mixed microbial communities are used as starter-cultures in many branches of food technology, in fodder production and waste waters processing *(1,2)*.

Their application is of major significance in milk processing, in the baker's trade, in meat and fish fermentation, pickled vegetables production, in alcoholic drinks production and fodder preservation. In practically all those industrial processes bacteria of lactic acid fermentation (BLAF) are present in starter-cultures. Their antimicrobial, anti-cholesterol and anti-cancerogenic properties have secured their use as therapeuticals *(3)*.

Biotechnologists have noticed the very important role played by the bacteria of the genus *Lactobacillus* in the fermentation of doughs for bread and roll production. It has been established that the use of *Lactobacillus* species in pure or mixed cultures with yeasts has a favourable effect on the quality of sour dough and bakery products *(4–10)*.

The application of these cultures in bakery is most frequent in the production of dark sour bread, rolls and sweet goods (brioches, pannetone). The fermentation of dough with bacteria of lactic acid fermentation improves the quality of the dough, i.e. the dough elasticity, which is manifested in a better structure of the bread crust, a more pronounced and aromatic taste, in increased shelf life and lower crumbling and staling of the bread *(11,12)*.

The role of lactic acid fermentation bacteria in dough souring

Many bacteria used nowadays in starter-cultures for sour dough fermentation have been isolated from dough made of rye or wheat flour *(13–18)*. A list of the bacteria of lactic acid fermentation most commonly used in starter-cultures for sour doughs and their main characteristics are given in Table 1.

Some bakeries still use natural microflora of flour for the dough fermentation by spontaneous souring. The dough prepared from flour and water is allowed to rest at a temperature of 26–35 °C for 2–3 days. The formation of gas and the sour smell of the dough mark the beginning of acid fermentation. After a few days, sour dough

Table 1. Lactic acid bacteria for sour dough fermentation *(19–21)*
Tablica 1. Bakterije mliječno-kiselog vrenja za fermentaciju kiselih tijesta *(19–21)*

Bacterial species	Morphology	Metabolic pathway of glucose fermentation
Lactobacillus acidophilus	rods	homofermentation
Lactobacillus plantarum	rods	homofermentation
Lactobacillus casei	rods	homofermentation
Lactobacillus delbrueckii	rods	homofermentation
Lactobacillus brevis	short rods	heterofermentation
Lactobacillus fermentum	rods	heterofermentation
Lactobacillus fructivorans	rods	heterofermentation
Lactobacillus sanfrancisco	long rods	heterofermentation

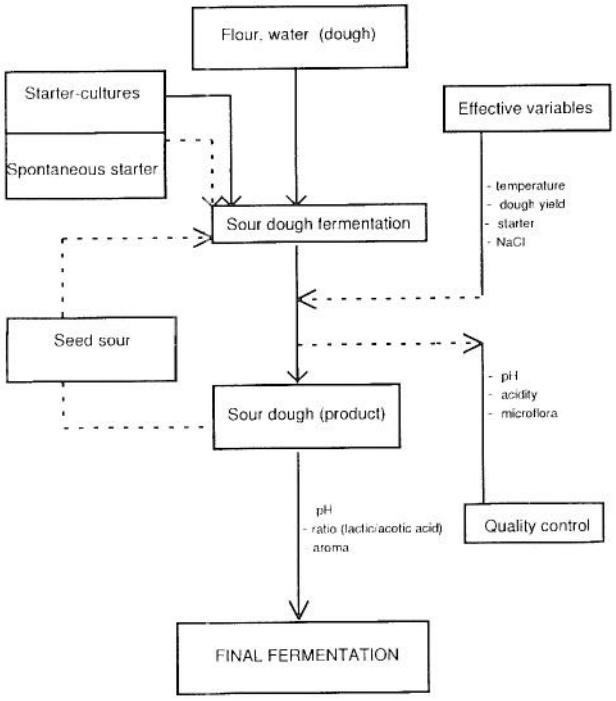

Fig. 1. Shematic presentation of the rye dough process using starter-cultures *(22)*

Slika 1. Prikaz proizvodnje raženog kruha primjenom mikrobnog cjepiva *(22)*

develops a degree of acidity of 14–20 and pH-value of 3.6–3.9. The microflora of such doughs contains from $3.7 \cdot 10^9$ to $7.5 \cdot 10^9$ yeast cells per gram *(22)*. The bacteria in such doughs mainly include lactic acid fermentation bacteria of the genus *Lactobacillus*, and the yeasts belong mainly to the genus *Saccharomyces* and *Candida*.

Production of rye bread with sour dough fermentation by commercial starter-cultures or by spontaneous starter is presented in Fig. 1.

Spontaneous starter of the dough sometimes leads to the presence of undesirable microorganisms in the flour, so that nowadays starter-cultures of a defined microbial composition are mainly used for sour dough preparation.

The most frequently listed homofermentative bacterium in dough souring is *Lactobacillus plantarum (23)*. However, for the formation of metabolite mixture (lactic acid, acetic acid, carbon dioxide), the heterofermentative bacteria of lactic acid fermentation are more important; the most commonly used among them are the bacteria *Lactobacillus brevis, Lactobacillus brevis* var. *lindneri, Lactobacillus reuteri, Lactobacillus sanfrancisco* and *Lactobacillus fermentum (22,24,25)*. Later on it was established that the bacteria *L. sanfrancisco* and *L. brevis* var. *lindneri* were genetically the same microorganisms.

In order to supply the producers of bakery products with active microbial cultures, it is of great importance to select an appropriate substrate for the cultivation of physiologically active starter-culture which, in the dough fermentation, lowers the pH to about 3.8 by forming lactic acid and acetic acid in a desirable ratio *(26)*.

Sensory qualities of baked goods are fully attained if mixed cultures of homofermentative and heterofermentative lactic acid bacteria are present in starter-cultures for sour doughs *(2,24,25,27–29)*. Bakery products obtained by dough fermentation with the heterofermentative bacteria *L. brevis* and/or *L. sanfrancisco* have the desired aroma but insufficient elasticity; if, however, the homofermentative bacteria *L. plantarum* and *L. farciminis* are used for dough fermentation, the final products will be lacking in aroma but elasticity and uniform porousness will be achieved *(30, 31)*.

The fact that the bacteria of lactic acid fermentation are indispensable is especially manifest in rye bread production. Rye bread is usually made from mixed flour (rye + wheat at a ratio of 30:70 to 50:50). It has been proved that by souring the rye-flour dough a good quality of bread is obtained, which is a result of increased enzymatic activity; the desired elasticity of the protein structure in the dough and the characteristic smell and aroma is developed (lactic and acetic acids, precursors of aromatic substances) during the dough fermentation *(1,32,33)*.

This sort of bread has a longer shelf life, crumbles less when cut and has a fine grain structure and aroma.

Bacteria of lactic acid fermentation are also used for the preparation of wheat sour doughs. Sour fermentation of wheat dough with bacteria enhances the quality of the bread, which is manifested in the following properties:

1. Improved viscous-elastic properties of the gluten showed in the texture of the crust. The crust structure of wheaten bread becomes softer, better bound and more elastic. The inner grain structure of the crumb has smaller pores, and a larger volume of bread is achieved *(32–34)*.
2. A more pronounced and aromatic taste *(33,34)*.
3. A better shelf life of baked goods *(35,36)*.

In order to increase the shelf life of baked goods, in addition to bacteria of lactic acid fermentation, starter-cultures often contain bacteria which form propionic acid. The presence of propionic acid in addition to lactic and acetic acids inhibits the growth of mould *(37)*. However, in order that the propionic acid bacteria can form a sufficient amount of propionic acid, their count in the dough must be at least $2.5 \cdot 10^8$/g of dough. Bread manufactured in this way (especially the brown type)

may have a long shelf life, which is of strategic importance in critical situations.

The ratio of lactic acid to acetic acid formed (90:10 to 70:30) is of greatest significance for a good quality of bread. In order to attain a satisfactory ratio of lactic acid to acetic acid in bread, it is necessary to determine the parameters (temperature, dough consistency) which are essential to achieving it.

The effect of temperature on the formation of acetates and lactates has been studied by acidification of the dough with heterofermentative lactic bacteria (*L. brevis* var. *lindneri*, *L. buchneri* and *L. fructivorans*). Depending on the temperature of dough fermentation, various amounts of lactic and acetic acids are produced. The formation of lactates by dough fermentation with the mentioned bacteria is optimal at higher temperatures (32–35 °C), while a lower temperature range (28–30 °C) is more favourable for acetate formation.

The effect of dough consistency (firmness), often called the dough yield, on the formation of acids has also been investigated with homofermentative and heterofermentative lactic acid bacteria. It has been established that the formation of acids decreases with a lesser firmness of the dough. Since the formation of lactic and acetic acids does not follow the changes in the dough consistency at the same ratio, greater or lesser changes occur in the quotient of the ratio of lactic acid to acetic acid *(32,37–40)*.

The role of yeasts

Although the bacteria of lactic acid fermentation play a certain role during the fermentation of the dough, the yeasts are mainly responsible for the rise of the dough *(41)*. The industrially produced yeast, mainly used by bakers, is *Saccharomyces cerevisiae*. Its use spread rapidly after World War II, when fast technological processes in bread production were introduced and the use of sour dough was practically abandoned. Still, after a certain time manufacture of brown bread with sour dough was resumed in order to improve its quality. Other yeasts of various genera began to be used in the production of special types of bread, rolls and sweet goods. A list of yeasts used in starter-cultures for the manufacture of sour dough and their main characteristics are presented in Table 2.

Table 2. Yeasts in starter-cultures for sour dough *(42,43)*

Tablica 2. Kvasci u mikrobnim cjepivima za kisela tijesta

Yeast species	Morphology	Optimal temperature °C
Saccharomyces cerevisiae	oval	30–35
Saccharomyces uvarum	oval	25–35
Torulopsis delbrueckii	spherical	30–32
Saccharomyces exiguus	elliptical	25–30
Torulopsis holmii	oval	22–30
Pichia saitoi	oval	30–32
Candida krusei	oval	30–32
Saccharomyces rosei	round	30–32
Saccharomyces rouxii	elliptical	30–32
Kluyveromyces fragilis	elliptical	35–37
Torulopsis candida	spherical	30–32

The most commonly used yeasts in starter-cultures for sour dough are: *Saccharomyces exiguus, Saccharomyces cerevisiae, Canida krusei, Saccharomyces uvarum, Torulopsis holmii* and *Pichia saitoi (44)*.

Torulopsis holmii, the non-sporogenic form of the yeast *Saccharomyces exiguus* is considered to be the most important of them. According to a relatively recent classification this yeast is known under the name *Candida milleri (45)*. It can coexist with the bacteria of lactic acid fermentation because it is tolerant to the acetic acid formed. It tolerates very well a low pH-value and is resistant to antibiotics produced by the bacteria. It should be pointed out that this yeast does not ferment maltose which is rapidly fermented by the bacterium *L. sanfrancisco*. In this way, the rate of dough acidification and the formation of a specific aroma are increased in mixed culture *(46)*. While the yeast *Saccharomyces cerevisiae* is used in the manufacture of all types of bread, rolls and sweet goods, *Saccharomyces exiguus* is most frequently used in the production of the »San Francisco« sour dough *(44)*.

The yeasts *Saccharomyces rosei* and *Torulopsis delbrueckii* are used for frozen fermented doughs and their products. Besides the yeast *Saccharomyces cerevisiae*, the yeast *Kluyveromyces fragilis* is employed in some countries for the manufacture of bread and sweet goods *(47,48)*.

The yeasts *Torulopsis cellulosa* and *Torulopsis candida* are used in starter-cultures for the production of »Sough« bread in Iran *(49)*.

Saccharomyces cerevisiae, Candida krusei and *Saccharomyces exiguus* are often used in Germany for the production of sour doughs *(4)*.

Candida lusitaniae and *Saccharomyces delbrueckii* are often used together with the yeast *Saccharomyces cerevisiae* in some countries in order to produce a better aroma of sour dough *(50)*.

A special place in baking industry is taken by yeast *Saccharomyces uvarum*. The use of this yeast is especially emphasized in the production of »fine rolls«. In addition to being osmo-tolerant, this yeast allows fermentation at lower temperatures (25 °C). Therefore, this yeast is being increasingly used in the countries with best bakery tradition, such as Italy and France.

Mixed microbial cultures for dough souring

The countries of Western Europe and the USA mainly use mixed starter-cultures for the acidification of the dough. The application of such starter-cultures and the various interactions between the microorganisms present accelerate dough fermentation and the aroma and the elasticity of bread. The above statements have been proved by series of trial baking.

A comparison of dough fermentation with bacteria and the fermentation with yeasts and bacteria has shown that a product obtained by dough fermentation with mixed cultures of bacteria and yeasts has much better organoleptic and physico-chemical properties *(25,35,50)*.

The quality criteria of sour dough and breads as a function of the starter-cultures are given in Table 3.

Table 3. Quality criteria of sour dough and breads as a function of starter-cultures *(22)*

Tablica 3. Utjecaj sastava mikrobnog cjepiva na kakvoću kiselog tijesta i kruha *(22)*

Starter-cultures	Sour dough		Bread quality										
	pH	Acidity	pH	Acidity	Elasticity of crumb					Flavour				
					1	2	3	4	5	1	2	3	4	5
L. plantarum	4.05	7.90	4.50	6.50				O			O			
L. fermentum	4.30	8.46	5.70	6.80		O						O		
L. casei	4.25	7.00	4.70	6.20			O				O			
L. brevis	4.20	4.40	4.40	8.00										
L. sanfrancisco	3.90	4.30	4.30	8.90					O				O	
Mixed cultures of bacteria and yeasts	3.80	4.30	4.30	8.50					O					O

In addition to bacteria of lactic acid fermentation, various strains of yeasts of the genera *Candida, Saccharomyces* and *Pichia* are present in mixed starter-cultures. The interaction between individual strains of yeasts and bacteria of lactic acid fermentation during dough acidification has been extensively studied, since some mixed cultures for sour dough contain several strains of yeasts, and their interaction as well as their impact on the bacterial population is not always the same.

The impact of the yeasts on the bacteria can be either antagonistic, synergistic or indifferent *(51)*. Depending on the specific interaction between bacteria and yeasts, the level of acidity in dough acidification is affected in different ways. *T. holmii* in mixed culture with bacterium *L. brevis* var. *lindneri* increases the level of acidity in dough, while the yeast *S. cerevisiae* only in a mixed culture with the bacteria *L. brevis* var. *lindneri* and *L. fermentum* has positive effects on the acidification during dough fermentation *(52)*.

The behaviour of homofermentative bacteria in a mixed starter-culture with yeasts has also been investigated. The studies involved mixed cultures of yeasts (*C. krusei, P. saitoi, T. holmii* and *S. cerevisiae*) and bacteria (*L. acidophilus, L. plantarum* and *L. farciminis*) in the fermentation of rye flour sour dough. Depending on the interaction between bacterial strains and yeasts, the growth of bacterial strains and yeasts and the acidity of the dough are affected in various ways. The growth of the bacterium *L.farciminis* is not affected by the growth of the yeasts *C. krusei* and *P. saitoi*. However, *T. holmii* and *S. cerevisiae* favour the growth of the bacteria *L. plantarum* and *L. acidophilus*. This effect is more expressed only when larger amounts of the yeast inoculum (from $5 \cdot 10^6$ to $1 \cdot 10^7$ yeast cells/g of sour dough) are used.

The yeast *S. cerevisiae* stimulates the growth of the bacterium *L. acidophilus*, decreasing, at the same time, the growth rate of the bacterium *L. plantarum*. The yeast *C. krusei* increases the acidity of the dough only when used in a mixed starter-culture with the bacterium *L. acidophilus*.

Other factors (firmness of dough, natural microflora of the flour, temperature) also have an impact on the interaction between sour dough bacteria and yeasts.

Thus, it has been established that the temperature and the firmness of the dough, as well as the right choice of the starter-culture have a considerable influence both on the formation of lactic and acetic acids and on the biosynthesis of other organic acids (butyric, valeric acid) which enhance the aroma of final products *(53,54)*. On the other hand, selected microbial communities (*S. cerevisiae* and *S. boidini; L. plantarum* and *Streptococcus* sp.) influence the enzymatic activity in the dough during dough acidification.

The amounts of sugars (glucose, fructose and maltose) are satisfactorily lowered, and variable changes occur in the formation of lactic and acetic acids. In this way, the baked goods have various sensory qualities *(55)*. The organoleptic properties of bread depend to a large extent on the chosen microorganism strains and on the amount of lactic and acetic acids formed.

Table 4 gives a list of mixed starter-cultures very often used for sweet goods and sour breads. However, many different combinations of bacteria and yeasts in mixed cultures are used in the production of bakery goods. As was mentioned above *S. uvarum* nowadays could be a favourable yeast either in a mixed culture with some bacteria or in pure cultures.

Table 4. Starter-cultures for the preparation of different bakery products

Tablica 4. Mikrobna cjepiva za pripravu različitih pekarskih proizvoda

Kind of product	Country	Type of starter-cultures
Pannetone	Italy	*L. brevis* spp. *lindneri* *S. cerevisiae* *Streptococcus* sp.
Bread (wheaten flour)	Switzerland	*L. brevis* spp. *lindneri* *L. brevis* *L. hindegardii* *C. milleri*
Bread (mixed flour) (wheat and rye)	Germany	*L. brevis*. spp. *lindneri* *L. acidophilus* *L. plantarum* *L. brevis* *Pichia saitoi* *C. krusei*
French bread (wheaten flour)	United States of America	*L. sanfrancisco* *S. exiguus* *T. holmii* *S. inusitus*
Produce of wheat flour	Netherland	*L. brevis* spp. *lindneri* *L. sanfrancisco* *S. exiguus*
Bread (wheaten flour)	Italy	*L. brevis* spp. *lindneri* *S. cerevisiae* *S. species*

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