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scientific note

The Influence of Temperature on Production of Organic Acids in Dough Fermentation with *Lactobacillus brevis*, *Saccharomyces uvarum* and *Candida krusei* (in Pure and Mixed Cultures)

Utjecaj temperature na nastajanje organskih kiselina u fermentaciji tijesta s *Lactobacillus brevis, Saccharomyces uvarum* i *Candida krusei* (u čistim i mješovitim kulturama)

S. Grba, Vesna Stehlik-Tomas, Đurđica Šehović and S. Romac
Faculty of Food Technology and Biotechnology, University of Zagreb

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Summary

The conditions of dough fermentation with **L. brevis** *bacteria in pure and mixed* **S. uvarum/C. krusei** *cultures have been explored in order to produce optimal quantities of lactic, acetic and other organic acids capable of improving dough properties. These acids give a more aromatic flavour to the bread, increase its durability and reduce bread crumbling and deterioration.*

It was found that dough souring with **L. brevis** *and yield 200 (dough and wheat flour ratio × 100) produced the maximal quantity of lactic acid when fermentation occurred at 30 to 35 °C (0.76 %). In these conditions the fermentation temperature had no influence on the production of acetic acid.*

The greatest concentrations of acetic and lactic acids were obtained with a mixed **L. brevis/C. krusei** *culture at 30 °C (0.9 % lactic acid, and 0.2 % acetic acid).*

The best results in the production of organic acids were achieved in dough fermentation that was carried out with a mixed **L. brevis/S. uvarum** *culture. When dough was soured at 25 to 35 °C, concentrations of i-butyric, valeric, i-valeric and unindentified acids, in addition to lactic acid (0.7 %) and acetic acid (0.2 %), have also been quantified.*

Sažetak

Istraženi su uvjeti fermentacije tijesta s bakterijom **L. brevis** *u čistoj kulturi i mješovitim kulturama s kvascima* **S. uvarum** *i* **C. krusei** *radi proizvodnje optimalne količine mliječne i octene kiseline te drugih organskih kiselina kojima se poboljšavaju svojstva tijesta. Navedene kiseline daju kruhu aromatičniji okus i produljuju mu trajnost te smanjuju mrvljenje i kvarenje.*

Utvrđeno je da je kiseljenjem tijesta s bakterijom **L. brevis** *pri iskorištenju 200 (odnos tijesta i brašna × 100) nastala maksimalna količina mliječne kiseline kad se fermentacija odvijala pri 30–35 °C (0,76 %). U tim uvjetima temperatura fermentacije nije utjecala na proizvodnju octene kiseline.*

Najveće koncentracije octene i mliječne kiseline dobivene su s mješovitom kulturom **L. brevis** *i* **C. krusei** *pri 30 °C (0,9 % mliječne kiseline i 0,2 % octene kiseline).*

Fermentacijom tijesta s mješovitom kulturom bakterije **L. brevis** *i kvasca* **S. uvarum** *postignuti su najbolji rezultati proizvodnje organskih kiselina. Naime, kiseljenjem tijesta pri 25 – –35 °C, uz mliječnu kiselinu (0,7 %) i octenu kiselinu (0,2 %), ustanovljene su koncentracije i-maslačne, valerijanske, i-valerijanske te neidentificiranih kiselina.*

Introduction

Bakers yeast, produced and used nowadays in fast continuous or semi-continuous bread and fancy bread production, does not provide the expected quality of final products. Therefore, the bakers yeast as a pure culture is gradually abandoned in baking industry, especially in Western Europe. For several decades special attention has been focused on starter cultures composed of yeast and lactic acid bacteria *(1–3)*. Mixed cultures of yeast and bacteria in dough fermentation produce CO_2, ethanol, lactic and acetic acids and other natural products of the biological process. They provide the specific aroma and acidity to the resulting product, increase its durability and reduce bread crumbling and deterioration *(4)*.

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It is also necessary to point out that the quality of baking products depends considerably on the microorganisms in starters and their physiological state. In many starter cultures used in the bakery, especially in Western Europe and USA, mixed cultures composed of lactic acid bacteria and different yeast strains have been used *(2,5)*. Many authors have stated that heterofermentative lactic acid bacteria have the dominant role *(1,2,5)*. On the other hand, the other parameters, such as temperature, dough yield and flour quality, have also great influence on the dough souring.

The goal of the research that had been carried out was to discriminate an appropriate starter culture and dough fermentation temperature, likely to produce the required quantity of lactic, acetic and other organic acids, which can improve the quality of bakery products.

Materials and Methods

The following yeasts and bacteria were used:

- dehydrated *L. brevis* culture No. 62, supplied by the CHR HANSENS laboratory (Denmark). The culture was reactivated and maintained in the MRS medium *(6)*;
- *S. uvarum* yeast isolated from sour dough, acquired from a private bakery in France (Paris);
- dehydrated *C. krusei* yeast culture, supplied by the Institute of the »Agricultural Research Service«, Peoria, USA. It was reactivated and maintained in a yeast substrate.

Yeast medium was prepared by dissolving 20 g of glucose, 10 g of bactopeptone, 5 g of yeast extract and 20 g of agar in distilled water, supplemented up to 1 L. The pH value of the solution was 6.5.

The *L. brevis* inoculum was cultivated in the modified liquid MRS medium in a thermostat at 37 °C during 24 hours.

The *S. uvarum* and *C. krusei* yeast inocula were cultivated in the liquid yeast medium in a shaker at 30 °C with 150 rpm during 24 hours.

Starters in pure and mixed cultures were cultivated in a mixture of wheat flour type 500 and water with 1:1 ratio (yield 200) at 20, 25, 30 and 35 °C during 20 hours.

Total acids in the fermented dough were determined by titration *(7)*, while the identification and quantification of organic acids was made by gas chromatography *(8)*. The samples used in gas chromatography were prepared by extracting 60–120 g of dough in a plastic bag with 250 mL of 0.05 M H_2SO_4 at 20 °C during 4 minutes.

Results and Discussion

The effect of temperature on organic acids production during 20 hours of fermentation with bacteria *L. brevis* is presented in Fig. 1.

As shown, the optimal concentration of lactic acid was achieved at 30 and 35 °C (0.7 %). The optimal concentration of acetic acid occurred at 20 °C. The results obtained are in agreement with earlier findings *(9,10)*. The referenced authors have shown that the production of lactic acid by lactic acid bacteria is more temperature-dependent than the production of acetic acid. It means that optimal temperatures for bacteria growth are not necessarily optimal for acetic acid formation.

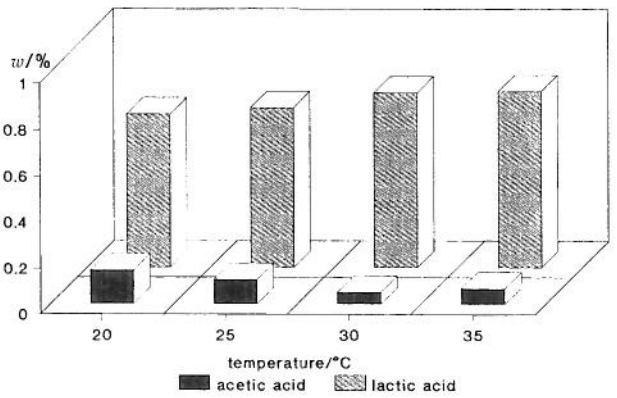

Fig. 1. The influence of temperature on production of lactic and acetic acids during fermentation with *L. brevis*
Slika 1. Utjecaj temperature na nastajanje mliječne i octene kiseline tijekom fermentacije s *L. brevis*

Fig. 2. shows the concentrations of lactic and acetic acids produced by the mixed *L. brevis/S. uvarum* culture, and Fig. 3. those of mixed *L. brevis/C. krusei* culture.

It is obvious that at 30 °C higher concentrations of lactic and acetic acids are obtained with mixed than with pure *L. brevis* culture.

Both yeast strains used in mixed cultures with *L. brevis* increased the production of lactic and acetic acid.

The results obtained with *L. brevis/C. krusei* at 30 °C are not in accordance with some other results *(10)* where it was found that *C. krusei* yeast increases lactic acid production only if *L. acidophilus* bacteria are present.

Although temperature has a major influence on the production of lactic and acetic acids, it should be pointed out that the process is also affected substantially by dough yield, flour type and dough pH value.

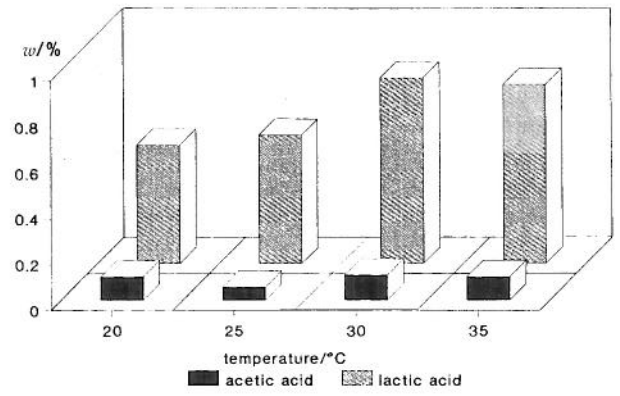

Fig. 2. The influence of temperature on production of lactic and acetic acids during fermentation with *L. brevis* and *S. uvarum*
Slika 2. Utjecaj temperature na nastajanje mliječne i octene kiseline tijekom fermentacije s *L. brevis* i *S. uvarum*

Table 1. Concentrations of organic acids produced during dough fermentation with *L. brevis* and mixed *L. brevis/S. uvarum* and *L. brevis/C. krusei* cultures
Tablica 1. Koncentracije organskih kiselina nastalih za vrijeme fermentacije tijesta s *L. brevis* i mješovitim kulturama *L. brevis/S. uvarum* i *L. brevis/C. krusei*

Cultures Kulture	Organic acids – Organske kiseline				t_C/°C
	$w\left(\frac{\text{i–butyric}}{\text{i–maslačna}}\right)/\%$	$w\left(\frac{\text{valeric}}{\text{valerijanska}}\right)/\%$	$w\left(\frac{\text{i–valeric}}{\text{i–valerijanska}}\right)/\%$	$w\left(\frac{\text{unidentif.}}{\text{neidentif.}}\right)/\%$	
L. brevis	0	0	0	0	
L. brevis/S. uvarum	0	0	0	0	20
L. brevis/C. krusei	0	0	0	0	
L. brevis	0	0	0	0	
L. brevis/S. uvarum	0.003	0.001	0	0.001	25
L. brevis/C. krusei	0.015	0	0	0.001	
L. brevis	0	0	0	0.004	
L. brevis/S. uvarum	0.01	0.001	0	0	30
L. brevis/C. krusei	0.02	0	0	0	
L. brevis	0	0	0.003	0	
L. brevis/S. uvarum	0.004	0.001	0.001	0	35
L. brevis/C. krusei	0.01	0	0	0	

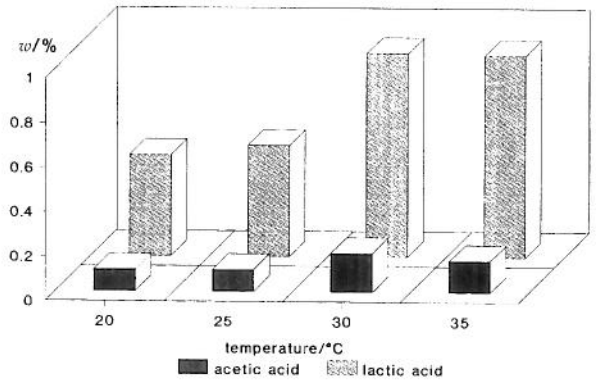

Fig. 3. The influence of temperature on production of lactic and acetic acids during fermentation with *L. brevis* and *C. krusei*
Slika 3. Utjecaj temperature na nastajanje mliječne i octene kiseline tijekom fermentacije s *L. brevis* i *C. krusei*

In our experiments, carried out under the described conditions, the greatest concentration of lactic and acetic acids have been obtained at 30 °C (dough yield 200) in mixed cultures with *L. brevis/C. krusei*. It seems that the temperature of 30 °C is optimal for the production of acids. These results coincide with those obtained by G. Spicher *(11)*.

Collar et al. *(12)* have found that some acidification properties (sour dough, pH, total titratable acidity of dough and bread) and sensory attributes (external appearance, typical flavour) as well as rheological parameters (fermentation time, degree of softening) depend on both the type of flour and physical conditions of the starter.

Concentrations of other organic acids produced in the course of dough fermentation with *L. brevis* bacteria, mixed *L. brevis/S. uvarum* culture and mixed *L. brevis/C. krusei* culture are presented in Table 1.

As can be seen, *L. brevis*, when used in fermentation individually, did not produce i-butyric nor valeric acid. But in mixed cultures with yeasts, it promoted an increase in the production of these acids. At 20 °C only lactic and acetic acids are produced. When the temperature is raised to 25, 30 or 35 °C, other organic acids are also generated. The highest concentration of these acids is obtained at 35 °C with mixed *L. brevis/S. uvarum* culture.

The results are in agreement with those obtained by Martinez-Anaya et al. *(13)*. These authors elaborated the changes in volatile compounds during dough fermentation with pure and mixed microorganisms. When lactic acid bacteria were used individually, they did not produce other organic acids. But when added to doughs with yeasts, the other organic acids were obtained.

Conclusion

One can conclude that dough fermentation temperature and selected culture are the main factors in the production of lactic, acetic and other organic acids. Dough fermentation with *L. brevis* and mixed cultures has shown that dough inoculation with mixed cultures enhances the production of lactic and acetic acids. By souring the dough with mixed *L. brevis/C. krusei* culture, maximal concentrations of lactic and acetic acids have been obtained, but other aromatic substances, those that improve the taste and flavour of the product, were not synthesised in sufficient quantities. When the dough was soured with mixed *L. brevis/S. uvarum* culture, the required concentrations of lactic and acetic acids and a wider spectrum of other organic acids were obtained. The dough inoculation with this mixed culture can improve the properties of the dough, namely its elasticity and flavour. It can also reduce bread crumbling.

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