УДК 664.642.1, 664.66.019

Use of a probiotic yeast strain in technology of bread from wheat flour*

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For the modern bakery is interesting to study yeast strains with good biotechnological properties and simultaneously antagonistic activity against pathogens of microbial spoilage of bread. The purpose of research — to study the effect of the probiotic strain of S. cerevisiae RCAM 01730 on the technological parameters of preparation of bread from wheat flour on the quality indicators of finished products and the suppression of rope in bread. Yeast antagonistic activity against spore-forming bacteria were determined by diffusion into agar. Immediate and total gas evolution in dough was evaluated using automatic risograph (USA). In order to detect signs of disease of rope in bread used method of placement the products in provoking conditions. The antagonistic activity of the strain of yeast S. cerevisiae RCAM 01730 with respect to the bacteria of the genus Bacillus was determined. The results of the research of the possibility of using a strain of Saccharomyces cerevisiae RCAM 01730 in wheat bread technology with sponge dough, straight dough, rapid dough methods were represented. It is shown that the use of a strain of S. cerevisiae RCAM 01730 instead of 02150 S. cerevisiae RCAM accelerating gas formation in the dough, intensifies the maturation process of the dough. The study found that a strain of the yeast S. cerevisiae RCAM 01730 has a bacteriostatic effect on the pathogens of potato disease of bread. In contrast to existing labor-intensive and time-consuming biological methods of combating microbial spoilage of bread, the use of the yeast S. cerevisiae RCAM 01730 can be an effective way to increase microbiological resistance without any additional costs.

Keywords: bakers' yeasts, *Saccharomyces cerevisiae,* rope in bread, antagonistic activity, gas generation.

Информация о статье
Поступила в редакцию 23.03.2016, принята к печати 24.10.2016
doi: 10.21047/1606-4313-2016-15-4-11-15
Ссылка для цитирования
Soboleva E. V., Sergacheva E. S., Ternovskoy G. V. Use of a probiotic yeast strain in technology of bread from wheat flour // Вестник Международной академии холода. 2016. № 4. С. 11–15.

Использование пробиотического штамма дрожжей в технологии хлеба из пшеничной муки

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Для современного хлебопечения представляет интерес изучение штаммов дрожжей, обладающих хорошими биотехнологическими свойствами и одновременно антагонистической активностью по отношению к возбудителям микробиологической порчи хлеба. Цель исследования — изучить влияние пробиотического штамма S. cerevisiae RCAM 01730 на технологические параметры приготовления хлеба из пшеничной муки, на показатели качества готовых изделий и на подавление картофельной болезни хлеба. Антагонистическую активность дрожжей по отношению к спорообразующим бактериям определяли методом диффузии в агар. Мгновенное и общее газовыделение в тесте оценивали с помощью автоматического ризографа (США). Для выявления признаков заболевания хлеба картофельной болезнью использовался метод закладки изделий в провоцирующие условия. Установлена антагонистическая активность штамма дрожжей S. cerevisiae RCAM 01730 отношению к бакте-

* This work was partially financially supported by the government of the Russian Federation, Grant 074-U01.

риям рода Bacillus. Представлены результаты исследований возможности использования штамма Saccharomyces cerevisiae RCAM 01730 в технологии пшеничного хлеба опарным, безопарным, ускоренным способами. Показано, что использование штамма S. cerevisiae RCAM 01730 взамен S. cerevisiae RCAM 02150 ускоряет газообразование в тесте, интенсифицирует процесс созревания теста. В ходе исследования установлено, что штамм дрожжей S. cerevisiae RCAM 01730 оказывает бактериостатическое воздействие на возбудителей картофельной болезни хлеба. В отличие от существующих трудоемких и длительных биологических методов борьбы с микробной порчей хлеба, применение дрожжей S. cerevisiae RCAM 01730 может служить эффективным способом увеличения микробиологической стойкости, не требующим дополнительных затрат.

Ключевые слова: дрожжи, *Saccharomyces cerevisiae,* картофельная болезнь, антагонистическая активность, газообразование.

Introduction

Microorganisms play a great role in breadmaking. Ethyl alcohol and lactic acid fermentation, of which the main causes are *Saccharomyces cerevisiæ* yeasts and lactic-acid bacteria *Lactobacillus*, both take place in wheat flour dough. However, besides fermentating microorganisms spore-forming bacteria of the genus *Bacillus* such as *B. subtilis*, *B. pumilus*, *B. licheniformis* can develop in prefermented and final products. These microorganisms can cause rope in bread.

The main infection sources during bread production are flour, bran and grain [1, 2]. Violation of processing procedures and poor sanitary conditions can be the cause of the finild product disease. The vegetative cells of *Bacillus* bacteria die during baking in the oven, but their spores are heat resistant and can germinate during bread storage under favorable conditions [3].

At the first stage of spoilage, the bread loses its native flavor and then specific sweet odor appears similar to that of overripe melon or valerian [4, 5, 6]. During spoilage, the odor intensifies and takes on a putrefactive character. The crumb becomes sticky; when the loaf is torn one can see slimy stretchy strings. Sometimes these thin silvery slimy web like strings stretch up to 50 cm. Hence the other name of this disease, the rope. In German such phenomenon is called «Fadenziehen des Brotes», in English, «Rope in bread», in Russian, «Potato disease». Crumb colour changes, blotches of yellow, brown and muddy pink appear. As the disease enters its final stage, bread turns into a dark slimy mass with a coarse odor and strong off-taste. The pores collapse, small holes and then larger ruptures appear in the crumb [4, 7, 8, 9].

Bacterial cells have active amylolytic and proteolytic enzymes. The proteins are broken down and amides, amines, amino acids and peptides appear. Starch is hydrolyzed into dextrin and mono- and disaccharides. The former contribute to bread crumb stickiness. Substances with a specific acrid odor are formed due to interaction of mono- and disaccharides with amines and amides. The amount of aldehydes and other compounds rises sharply which contributes to the rotten smell of the diseased bread [5, 6]. Consumption of bakery products affected by rope can lead to poisoning by metabolites accumulated in them such as toxins and degradation products.

The main factors that inhibit the development of rope in bread and corresponding pathogens are high acidity, low humidity, increased content of sugar and fat in the product (15–20% of the total weight of flour), the antibiotic activity of the medium. In this regard, bakeries use different methods, devices and techniques to fight rope in bread at all stages of manufacturing. There exist various methods for preventing the development of microbial spoilage of bakery products, chemical, physical and biological.

The most effective methods of rope control are biological methods, such that involve liquid yeast, sourdough and starter cultures. In recent years, there is significant demand for research of yeast strains capability to fight microbial spoilage of food substances of microbial origin [10]. Microorganisms of starter cultures used in baking are able to synthesize substances inhibiting exogenous microflora development in final products. The antagonistic activity of these microorganisms is due to accumulation of their metabolic products in semi products. The main batch of active metabolites is built up by lactic acid bacteria, which produce an array of natural antibacterial substances, including organic acids, carbon dioxide, hydrogen peroxide, diacetyl, ethanol, bacteriocins, reuterin, reutericyclin [10–14]. As to the yeast, their antagonistic role is not normally considered. However, in 1909 F. Hayduck published data on yeast proteins (killer factors) being toxic to other strains [15].

Antagonistic activity of yeast is caused by competition for nutrients, medium pH changing due to the ion equilibrium shift or formation of organic acids, production of large quantities of ethanol, release of antibacterial and antimicrobial substances, such as killer toxins — mycocins [16, 17]. Mycocins are extracellular proteins, mainly glycoproteins, that inhibit normal cell membrane functioning (integrity, osmotic permeability) in yeasts that have receptors with corresponding sensitivity [16]. Mycocin activity is mainly targeted at yeast cells genetically closely related to the producer strain, serving as a protective factor.

The yeast *S. cerevisiæ* synthesize several types of killer factors, some of which attach to β-1,3-glucan chains, opening channels in the cell membrane of sensitive strains, and others attach to mannoproteins of the cell wall and inhibit DNA synthesis [18].

Mycocin production occurs not only in *Saccharomyces* yeasts but also in *Candida*, *Cryptococcus*, *Debaryomyces*, *Kluyveromyces*, *Pichia*, *Torulopsis*, *Williopsis* and *Zygosaccharomyces* [16, 17]. However, not much research was focused on the effect of killer factors on bacterial microflora.

It is well known, that the development of spoilage microorganisms has a direct impact on final product quality. Therefore adding antagonistic yeast starter cultures is bound to enhance product safety by inhibiting pathogenic microorganisms growth during fermentation and final product sensory qualities and shelf life by inhibiting spoilage flora development [4–7, 19, 20].

Saccharomyces cerevisiae yeasts are the principal microorganisms in bread production, and as such, they show promise as an innate antagonist to bacteria *Bacillus spp* that cause rope in bread.

Materials and methods

Yeast strains *Saccharomyces cerevisiae* RCAM 02150 and *S. cerevisiae* RCAM 01730, bacteria strains *Bacillus subtilis* KM and *B. licheniformis* 1, dough, wheat bread.

Antagonistic activity of yeast in relation to spore-forming bacteria was estimated by the method of diffusion into agar [20].

Bread production and storage tests. Yeast strains *Saccharomyces cerevisiae* RCAM 02150 and *S. cerevisiae* RCAM 01730 used in the experiment were grown in pilot plant conditions subject to all parameters and stages applied in pressed bakers' yeasts production on the industrial scale.

The bread doughs were obtained by kneading 1,000 g of wheat flour (labeled «premium» according to the Russian classification) and 560 g of tap water in a mixer (Kitchen Aid 5KPM5 Professional, St. Joseph, Mich.) for 5 mins at room temperature. Yeast inoculum added at this stage was such that resulted in about 1.0×10^9 CFU g^{-1} (1%), 2.5×10^9 CFU g^{-1} (2.5%), 4.0×10^9 CFU g^{-1} (4.0%) viable yeast cells in the resulting doughs. After mixing, the doughs were shaped into roughly 400 g loaves, placed in aluminum pans, and leavened at 35 °C until the volume was twice the initial volume. The leavened doughs were baked in an oven at 210°C for 25 min. After cooling, the samples were stored at 37°C, 90% humidity (provocative conditions). In 24 hours, the loaves were examined externally for the presence of the typical sweet fruity odor, which characterizes the first stage of rope disease. The breads that gave off an intense smell were cut into halves and their crumb was inspected.

The volume of gas produced by different strains during dough fermentation was measured by rizograph test fermentation (National Manufacturing). Dough made as described above was rolled into balls and left to ferment for a maximum of 240 min at 30 °C in the rizograph test chamber. Gas volume was measured continuously at 1 min intervals [21].

Results and discussion

Strain *S. cerevisiae* RCAM 01730 was selected through the screening of yeast strains from different microorganism collections based on its antagonistic activity and gas production capacity.

The purpose of the research was to investigate the impact of *S. cerevisiae* yeast microorganisms on development of bacteria causing rope in bread, following the steps listed below:

— define antagonistic effect of *S. cerevisiae* on bacteria causing rope in bread using a solid nutrient medium, and then by putting test baked bread under provocative conditions;

— determine the effect of *S. cerevisiae* on the formation of carbon dioxide in the dough, the dough-making duration and the quality of the final products.

Control samples were prepared using *S. cerevisiae* RCAM 02150 yeasts widely used in the baking industry.

The antagonistic activity of yeast was detected by the method of diffusion to meat-peptone agar, *Bacillus subtilis* KM and *B. licheniformis* 1 were used as a testing culture. It is determined that culture liquid supernatant of the strain *S. cerevisiae* RCAM 01730 inhibits the growth of bacteria of the genera *Bacillus* in the lunulas. On the other hand, culture liquid supernatant of the strain *S. cerevisiae* RCAM intensifies bacterial growth. It allows to formulate an assumption about the presence of antagonistic activity of *S. cerevisiae* RCAM 01730 against bacteria of the genera *Bacillus*. This strain could be used for preventing of rope spoilage of bread during storage.

The main yeasts processing behavior is gas-production property resulting from enzyme activity. The maltase and zymase activities, dough fermentation property and osmo sensitivity have been determined. The tested yeasts have the higher zymase and maltase activities and dough fermentation property than the control sample by 17%, 15% and 25% correspondingly.

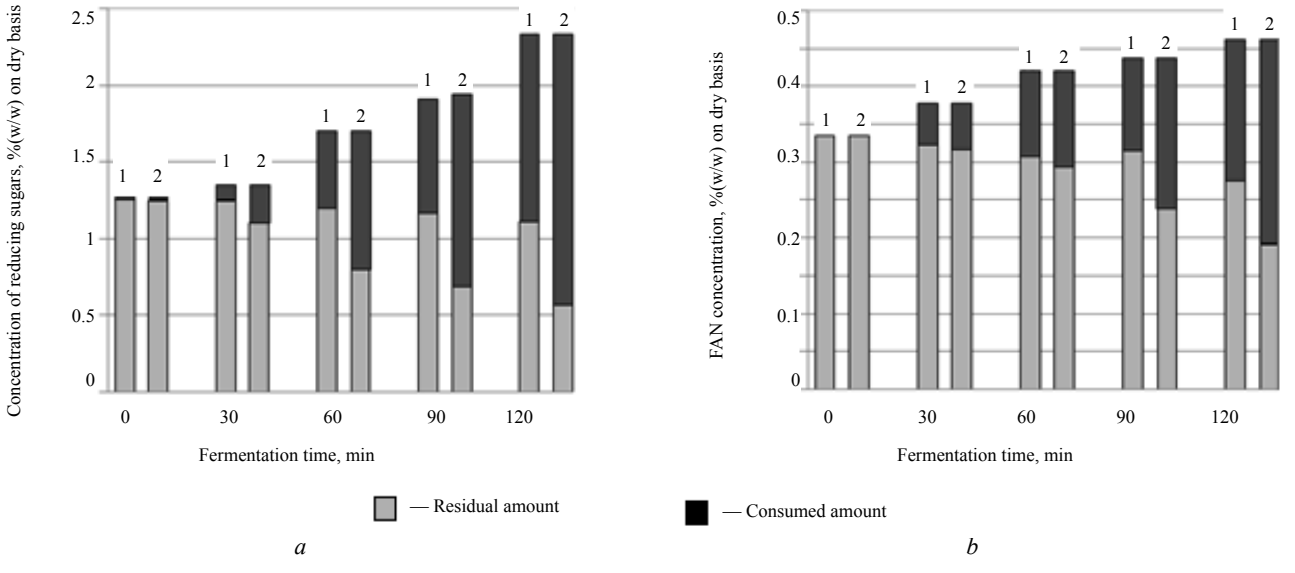

Figure 1. Changes in the content of reducing sugars (a) and FAN (b) in the dough with S. cerevisiae RCAM 02150 (1) and S. cerevisiae RCAM 01730 (2)

Yeasts fermentation activity influenced on dough maturation time and finished product quality has been estimated by total volume of released gas, the instant gas-production, amount of residual sugars and free α-amine nitrogen FAN in the dough.

The carbon dioxide production intensity was determined in dough samples prepared with the using of yeasts in dosages 1.0%, 2.5% and 4.0%, which correspond to sponge and dough method, straight dough bulk fermentation and rapid processing. Yeasts strain *S. cerevisiae* RCAM 01730 (dosage 4.0%) increase volume of carbon dioxide by 15% during 120 min of fermentation, then 2.5% during 180 min gives more by 18%, 1.0% during 240 min- more by 21% in comparison with yeasts *S. cerevisiae* RCAM 02150 shown.

Due to the action of amylolytic enzymes the reduction of sugars and FAN utilization by microorganisms occurs during the fermentation.

Studying changes in the content of reducing sugars and the FAN during the dough fermentation (Fig. 1), showed samples prepared with the yeast *S. cerevisiae* RCAM 01730, had intensive decrease of reducing sugars and FAN. In two hours of fermentation, with RCAM 01730 number of fermented sugars was more than 21%, and consumed FAN — 17% compared with the control.

Thus, the results of the study showed that the yeast strain *S. cerevisiae* RCAM 01730 are not inferior to strain RCAM 02150 in respect of biotechnological properties, therefore, these yeasts can be used for bread-production.

The influence of yeasts strain *S. cerevisiae* RCAM 01730 on the maturation time, the characteristics of pre fermented products under different technologies, the total time of dough-making and the bread quality has been estimated. The dough was prepared from baker's top-grade wheat flour by rapid processing, straight dough fermentation and sponge and dough method (big tight and liquid sponges were used) with such dosages of yeasts as 4.0%, 2.5% and 1.0% correspondingly. The dough prepared with yeasts *S. cerevisiae* RCAM 02150 was used as a control.

Experimental yeast allows to reduce dough fermentation and proofing the dough pieces (Table 1).

For example, by using the yeast *S. cerevisiae* RCAM 01730, the total duration of the dough development reduced by 8%, 10%, 14% and 22% by sponge and dough method (big tight and liquid sponges), straight dough fermentation and rapid methods respectively in comparison with using the yeast *S. cerevisiae* RCAM 02150. Thus, bread made by using strains of the yeast *S. cerevisiae* RCAM 01730 is not inferior to the control physical and chemical parameters (Table 1).

The sensory analysis (Fig. 2) showed that experimental samples have developed a thin wall porosity and have a more pronounced pleasant smell and taste.

It is determined that the use of yeast *S. cerevisiae* RCAM 01730 inhibits appearance of signs rope in bread.

Test laboratory baking of wheat bread with these yeast were made. The dough was prepared by sponge dough method, straight dough method and quick dough method with the yeast dosage yeast respectively 1.0, 2.5 and 4.0%. Bread was stored in provoking conditions — temperature 37 ± 1°C and relative humidity of 90%. The appearance of signs rope in bread were estimated organoleptically (Table 2).

According to the research results it is possible to conclude that the strain *S. cerevisiae* RCAM 01730 has the antagonistic activity against the bacteria *B. subtilis*, *B. licheniformis*.

Conclusions

During our research we have ascertained that yeast strain *S. cerevisiae* RCAM 01730 has a bacteriostatic influence on causative agencies of rope in bread. Usage of the strain *S. cerevisiae* RCAM 01730 instead of *S. cerevisiae* RCAM 02150 accelerates gas production in dough, intensifies process of dough maturation and improves organoleptic properties of bread quality.

Table 1

Effect of strains of the yeast S. cerevisiae to process parameters, quality parameters of semi-finished and final products

Parameters and indicators		The values of parameters at different methods of dough developing							
		sponge and dough method				straight dough		rapid	
		big tight sponges		liquid sponges					
		Strains of *S. cerevisiæ* RCAM yeast							
		02150	01730	02150	01730	02150	01730	02150	01730
Humidity, %	Pre-ferm.	41.5	41.5	68.9	68.8	—	—	—	—
	Dough	42.2	42.3	42.1	41.9	42.1	42.3	42.5	42.5
Acidity, deg	Pre-ferm.	3.2	3.5	2.8	3.0	—	—	—	—
	Dough	2.8	3.0	2.6	2.8	2.6	2.8	—	—
Fermentation duration, min	Pre-ferm.	210	210	240	240	—	—	—	—
	Dough	40	30	70	60	150	135	25	25
Proofing duration, min		80	65	90	60	65	50	90	65
Total duration of dough-making, min		330	305	400	360	215	185	115	90
Crumb humidity, %		41.5	41.5	41.5	41.5	41.5	41.5	41.5	41.5
Crumb acidity, deg		2.0	2.0	2.0	2.0	1.8	1.8	1.6	1.8
Porosity, %		73	78	74	80	72	78	69	72
Specific volume, sm^3 / g		3.35	3.60	3.45	3.65	3.25	3.60	2.90	3.20
Shape stability		0.52	0.55	0.50	0.54	0.53	0.56	0.52	0.54

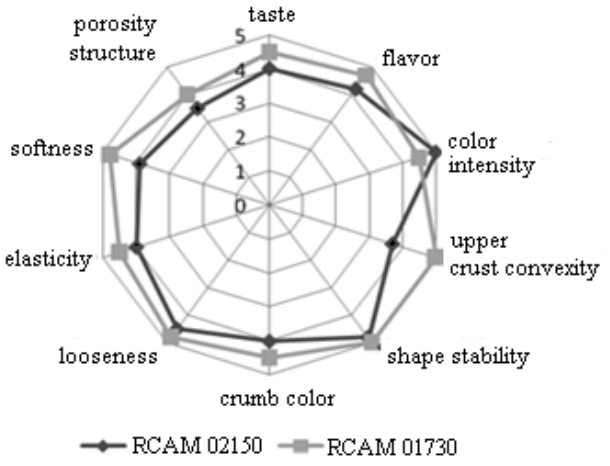

Figure 2. Organoleptic quality indicators of bread made by straight dough method using different yeast strains

Table 2

Influence of yeast strains on development of microbiological spoilage during the bread storage

Duration of storage, hours	Dough-making method					
	sponge dough method		straight dough method		rapid dough method	
	Saccharomyces cerevisiae RCAM					
	Strain 02150	Strain 01730	Strain 02150	Strain 01730	Strain 02150	Strain 01730
Development of rope in bread						
24	–	–	+	–	+	–
48	+	–	++	+	++	+
72	++	+	++	++	++	+
96	+++	++	+++	++	+++	++

"–" — no signs of microbiological spoilage;
"+" — initial signs of microbiological spoilage;
"++" — middle state of microbiological spoilage;
"+++" — intense microbiological spoilage

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