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ASSOCIATED CULTURES OF LACTIC ACID BACTERIA AND YEASTS IN THE INDUSTRIAL PRODUCTION OF BREAD

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Attempts were undertaken to intensify the fermentation process of industrial bread starters by the enrichment of the environmental microflora with the use of an associated population of *Lactobacillus sanfrancisco* and *Saccharomyces cerevisiae*. As a result, an equivalent relation bacteria/yeasts in a weekly cycle of starters restocking was obtained. The medium which ripened with the participation of pure cultures were characterized by a ca 10% higher level of lactic acid than in the case of spontaneously fermenting starters. The obtained bread had very good organoleptic properties; its standard indices were superior to those of bread manufactured according to the traditional technology.

INTRODUCTION

From among the non-raw material factors affecting the quality of rye and rye-wheat bread, the way of running the fermentation course of bread starters belongs to the most important [3, 16, 19, 22, 24].

In traditional technological conditions, this process has a spontaneous course as a result of the activity of the developing population of lactic acid bacteria and yeasts. The changes occurring in the starters, and especially the accumulated lactic acid, are favourable to the gelation of starch [41] improve the baking properties of pentosans and proteins of flour, make the dough more elastic and capable of retaining carbon dioxide produced by yeasts [28]. Biological acidification enriches bread [19] with readily assimilable protein compounds, B-group vitamins [31] and substances giving an aroma to bread. From among the active bacteria in starters, the main group is constituted by strains from the *Lactobacillaceae* family and among them, homo- and heterofermentative bacilli *Lacto-*

bacillus [22, 23, 24, 26, 30, 42]. Less frequent is the occurrence of *Streptococcus*, *Pediococcus* and *Leuconostoc* [16, 21]. Yeast are represented by a smaller number of species from which *Saccharomyces cerevisiae*, *Torulopsis holmii* and *Candida krusei* [18, 32, 36] are considered to be the most important. The majority of starter yeasts does not compete with lactic bacteria for the same sources of carbohydrates [21] moreover [35, 36, 44] certain products of metabolism as well as flour components create conditions for a symbiotic coexistence of both physiological groups. A condition of obtaining bread of good quality is an equilibrated lactic-ethanolic course of fermentation of bread starters [25, 34].

Particularly under the industrial conditions of bread manufacture, this purpose is often achieved by an enrichment of the natural starter microflora with pure cultures of active lactic bacteria [5, 15, 22, 34, 38].

In the studies undertaken on an appropriate orientation of the fermentation process of industrial bread starters, the associated cultures of *Lactobacillus sanfrancisco* and *Saccharomyces cerevisiae* were utilized.

EXPERIMENTAL

MATERIALS AND METHODS

The biological material used for the experiments were strains *Lactobacillus sanfrancisco*, isolated from loose starters [44]. Identification was carried out according to Kline et al [14], Sriranganathan et al [33] and on the basis of comparative tests with strains *Lactobacillus sanfrancisco* 119 and 120 from the collection of the Department of Food Science and Applied Microbiology of the University of Strathclyde; *Saccharomyces cerevisiae* A-1, isolated also from bread starters [42].

CULTIVATION MEDIA

The applied medium had the following experimental composition: Włodarczyk [45] rennet whey (lactose content 43.0-45.5 g/l) — 0.460 l, malt extract (concentrated malt wort with sugars level 432 g/l) — 75 g, sodium citrate — 5.0 g; technical glucose — 5.0 g; tap water in a supplementing quantity to 1 litre; pH = 6.2, conditions of sterilization: 30 min. 0.8 atm.

CONDITIONS OF CULTIVATION

A 20-l medium was inoculated with 24-h cultures of *Lactobacillus sanfrancisco* (7.5%) and *Saccharomyces cerevisiae* (5%), and incubated at 28°C for 24-26 h. The multiplied biomass of the associated population of

lactobacilli and yeasts together with the medium was added (10% capacity of leaven) to the so-called semi-sour (terminology connected with the fermentation process of leaven for production of wheat-rye bread was adopted as factory standard ZN-67 (MHW-PL-1) — phase initiating the weekly production of bread. Preparation of semi-sour initiated the preliminary cycle of fermentation: semi-sour — sour — loose leaven I — loose leaven II — sour — dough, lasting about 50 hours, called here after a 50-hour cycle. The actual production of bread during the 5-day period was performed by the 3-phase method: loose leaven — sour — dough, lasting for about 22 hours, called here after the production cycle.

MICROBIOLOGICAL ANALYSIS OF BREAD LEAVENS

The following determinations were made: total count of yeasts on medium malt wort 10°Blg and the level of acidifying bacteria in the modified Blickfeldt medium, by the method of plate inoculation [2]. Incubation was carried out at 28°C for 48 h. The results were given in units of the number of growth-capable microorganisms (CFU — colonies forming units), in relation to 1 kg of the environment.

DETERMINATION OF THE GROWTH PARAMETERS

Maximal specific rate of growth of yeasts and acidifying bacteria (u_{max}), the periods generation for both strains and the duration of the lag phase were calculated from the growth curves, illustrating the relationship $\log CFU = f/t$ [7].

CHEMICAL ANALYSES

Total acidity was determined by the titration method with 0.1 N NaOH vs. phenolphthalein. The results were related to 0.01 kg of the medium. Volatile acidity was determined by the distillation method with water vapor followed by titration with 0.1 N NaOH [8]. The results were given in g of acetic acid/0.1 kg medium. Lactic acid was determined by the colorimetric method [1, 9]. The results were given in g lactic acid/0.1 kg medium.

Technical analysis and score assessment of wheat-rye bread. The moisture level, volume and acidity of bread were determined according to the standard (PN-79/A-74108). Compressibility and relaxation were evaluated by the penetrometric method, using penetrometer type AP 4/2. The rate of penetration was expressed by the depth to which the dip rod penetrated the examined sample of bread after 60 and 120 secs. The results were given in degrees of penetration (1° corresponded to a penetration of the rod at a depth of 0.1 mm).

CALCULATIONS AND PRESENTATION OF THE RESULTS

The method of mathematical statistics was utilized [4] to elaborate the results of the experiments. A calculation was made of the coefficient of variability:

$$V_x = \frac{S_x}{\bar{x}}$$

where

S_x — mean standard deviation

\bar{x} — arithmetical mean of variable x

The number of determinations of series (n) in the presented experiments was 30 (50-hour cycle) and 60 (production cycle). The experiments were carried out in two industrial baking plants in Łódź, with manufacturing capacities of 40 tons bread/24 h.

THE RESULTS

The analysed bread starters in the preliminary 50-hour cycle as well as those related to the 3-phase industrial fermentation were characterized by correct organoleptic properties complying with the standard requirements. In starters from traditional production, however, a more intensive yeast — acetic aroma could be felt than in samples fermented with the participation of pure cultures. Direct microscopic observations revealed the presence of yeasts morphologically similar to *Saccharomyces* and of shorter rods; they were considerably more numerous in preparations of starters which were introduced from the semi-sour inoculated additionally with the associated population of *L. sanfrancisco* and *S. cerevisiae*. The results of quantitative determinations of both active groups of microorganisms confirmed these observations. The number of acidifying bacteria, capable of growth, in the preliminary fermentation period (Fig. 1) as well as in the starters of the production cycle (Fig. 3) was higher by three orders of magnitude in samples ripened with the participation of pure cultures. The level of yeasts (Fig. 2, 4) was also higher in these starters, however it did not exceed the 2-3 — fold number of cells found in the spontaneously fermenting media. As a result of additional inoculation of starter limiting the weekly cycle of fermentation with the use of associated population of *L. sanfrancisco* and *S. cerevisiae*, a far-going shift in the bacteria/yeasts relationship took place, i.e. from few hundred or even more than one-thousand-fold superiority of yeasts over acidifying bacilli in loose starters fermenting spontaneously to the equilibration system, or with a small superiority of 1-2 cells of bacteria/1 cell of yeasts. The last but one phase of fermentation (sour), according to the obligatory industrial formula, is enriched with a considerable addition of commercial yeasts

1.0-1.6 kg/100 kg acid. As a consequence of this operation, in spontaneously fermenting samples, the superiority of yeasts over bacteria reached the order of 10^4 . In sours and doughs having a share of pure cultures, this ratio was 0.2 : 1, i.e. there were 5 yeast cells per one bacterial cell capable of growth. The taking into account of initial and final CFU values for the particular phases of fermentation for both groups of microorganisms (Fig. 1-4) determined the areas of variability in the quantities of bacteria and

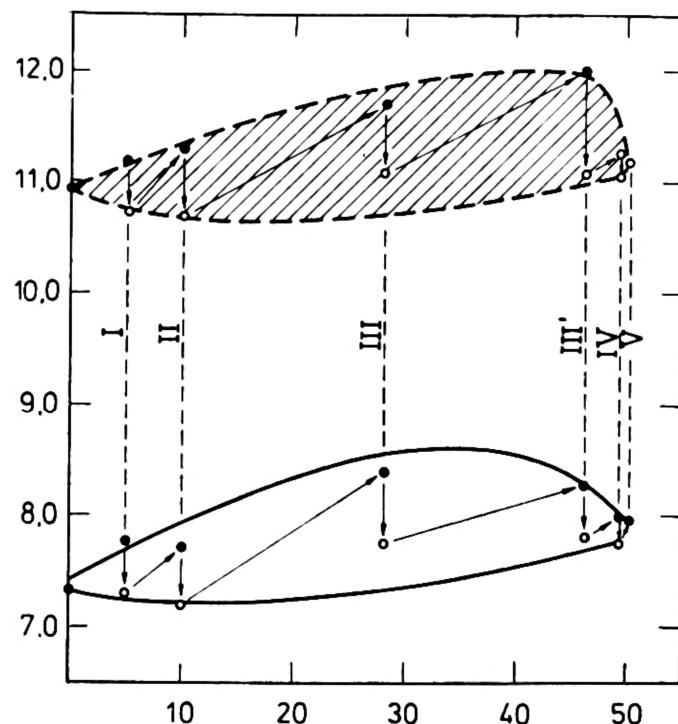


Fig. 1. Level of acidifying bacteria in 50-hour cycle of fermentation of bread starters (initiating the weekly production). Spontaneous fermentation ($n = 30$, $V_x = 0.09-0.12$). Fermentation with the participation of associated population of *Lactobacillus sanfrancisco* and *Saccharomyces cerevisiae* A-1 ($n = 30$, $V_x = 0.09-0.12$); I-V phases of fermentation, Y — axis: acidifying bacteria (log. CFU/kg) X — axis: (h)

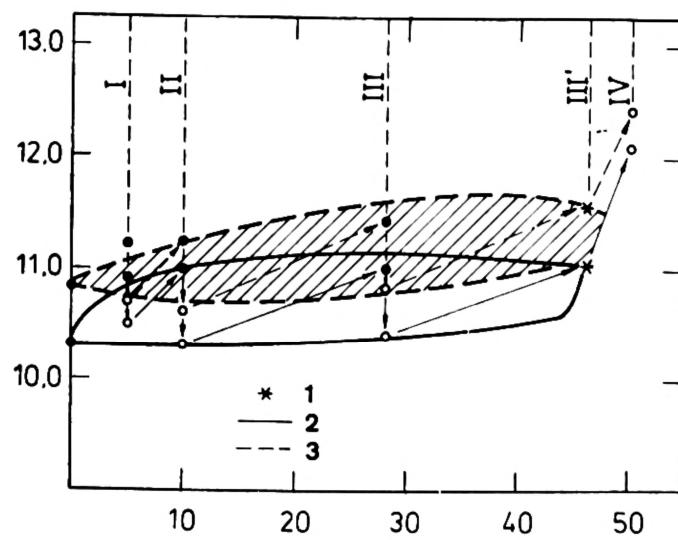


Fig. 2. Level of yeasts in 50-hour cycle of fermentation of bread starters (initiating the weekly production). Spontaneous fermentation ($n = 30$, $V_x = 0.09-0.11$) Fermentation with the participation of associated population of *Lactobacillus* and *Saccharomyces cerevisiae* A-1 ($n = 30$, $V_x = 0.09-0.11$); I-V phases of fermentation, x supplement of commercial pressed yeasts anticipated by the recipe, Y — axis: yeasts (log CFU/kg), X — axis: (h)

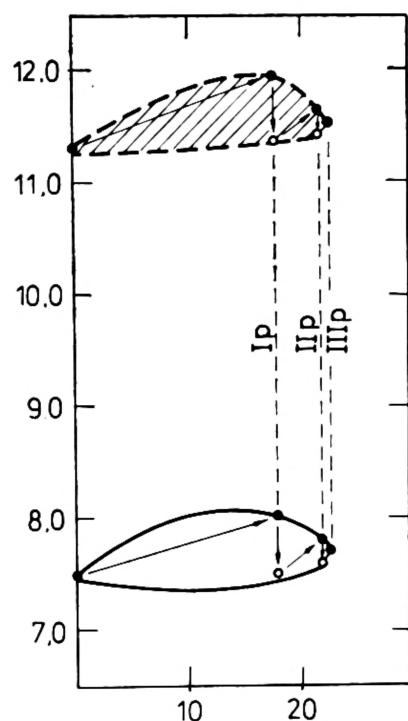


Fig. 3. Level of acidifying bacteria in three-phase (production) cycle of fermentation of bread starters. Spontaneous fermentation ($n = 60$, $V_x = 0.10-0.15$). Fermentation with the participation of associated population of *Lactobacillus sanfrancisco* and *Saccharomyces cervisiae* A-1 ($n = 60$, $V_x = 0.10-0.12$); I_p — III_p —phases of fermentation of production cycle, Y—axis: acidifying bacteria (log. CFU/kg), X—axis: (h)

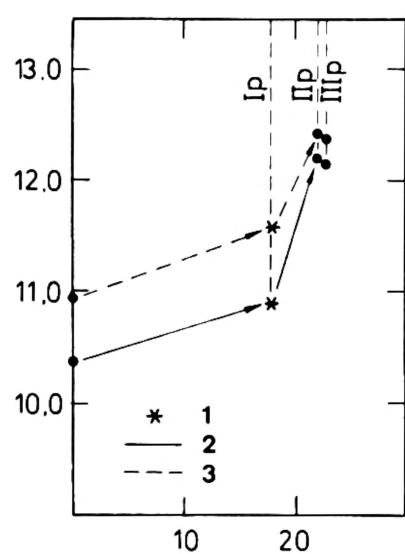


Fig. 4. Level of yeasts in three-phase (production) cycle of fermentation of bread starters. Spontaneous fermentation ($n = 60$, $V_x = 0.11-0.15$). Fermentation with the participation of associated population of *Lactobacillus sanfrancisco* and *Saccharomyces cerevisiae* A-1 ($n = 60$, $V_x = 0.10-0.13$); I_p — III_p —phases of fermentation of production cycle, x—addition of commercial pressed yeasts envisaged in the recipe, Y—axis: Yeasts (log. CFU/kg), X—axis: (h)

yeasts population. The highest number of acidifying bacteria cells of the order of $10^{11}/1$ kg was found during the final hours of fermentation of loose leavens, taking place with the participation of pure cultures. In comparison with the spontaneous process, these values were ca 7000 times higher.

The positive effect of additional inoculation was also reflected in the quantity of yeasts (Fig. 2, 4). The obtained multiplication points to the possibility of a considerable (by about 30%) limitation of the recipe addition of pressed yeasts. The conditions of the experiment, did not differ from the normal production course. In the manufacturing hall, several-degree differences in temperature level were recorded. The raw materials used were heterogeneous in terms of quality. Small break-downs and shut-downs took place. In this situation, the mean standard deviation for the performed analyses was relatively high, and the resulting coefficient of variability (V_x) ranged within 0.09-0.16. The acid-forming activity of the introduced starters, bacilli *L. sanfrancisco* was comparatively evaluated in relation to the natural bacterial microflora, on the basis of the accumulated products of metabolism. Total acidity complied with the standard requirements for bakery semi-products and those produced with the participation of pure cultures (Table 1) for sours and doughs, i.e. 7.9-8.8 and 7.3-8.2 ml 0.1 N NaOH/0.01 kg respectively. In loose leavens, this index ranged within 12.3-13.1 ml 0.1 N NaOH/0.01 kg.

In the total group of acids, the concentrations of its main components, lactic acid and volatile acids, were different. In starters fermented with the participation of pure cultures, lactic acid constituted 83-87% of total acidity. In self-fermenting samples, it did not exceed 76% of its value. The share of volatile acids was supplementary in relation to these values. In absolute values, concentration of volatile acids in terms of acetic acid in the spontaneously fermenting samples was 1.4-1.6 times higher than their level in starters with a participation of associated population of *L. sanfrancisco* and *S. cerevisiae*.

The share of the associated population of *L. sanfrancisco* and *S. cerevisiae* in the fermentation process of loose leavens (production phase I) was expressed by a shorter adaptation time bith of the population of bacteria and of yeasts by 30-60 min in comparison with the values obtained for natural microflora. In the samples fermented with the participation of pure cultures, there was a high agreement between the specific growth rates (μ_{max}) and the resulting periods of generation of yeasts and bacteria, and these results were maintained during the 5-day cycle of restocking and ripening of loose leavens.

The organoleptic evaluation of the final product was much more favourable for bread obtained from starters fermented with the participation of associated population of *L. sanfrancisco* and *S. cerevisiae*. It concerned both the shape of the loaf, appearance of crust, colour and structure of crumb (Fot. 1, 2) and flavour of bread.

In the 30-score evaluation, the maximal note was obtained whereas for bread manufactured by the traditional method, the result was expressed by 23-24 scores. Similarly, in other standard indices (Table 3) the moisture level was higher by 1.2% and the volume of bread was by ca 6%

Table 1. Total acidity, volatile acidity and level of lactic acid in fermentation cycle of bread starters in the spontaneous process and that with the participation of associated population of *L. sanfrancisco* and *S. cerevisiae* A-1

Phase of fermentation	Time of fermentation (h)	Spontaneous fermentation					Fermentation with the share of associated populations					
		total acidity		Lactic acid		volatile acids		total acidity		lactic acid		
		m 10,1 N NaOH	g 0,01 kg	g 0,1 kg	share in total acidity %	g CH ₃ COOH 0,1 kg	share in total acidity %	m 10,1 N NaOH 0,01 kg	g 0,1 kg	g 0,1 kg	share in total acidity %	
Preliminary cycle	I (Semi-sour)	5	12.4	0.83	74.5	0.18	24.1	11.8	0.82	77.2	0.16	22.6
	II (Sour)	5	12.2	0.82	74.9	0.18	24.2	12.0	0.92	85.2	0.12	16.7
	III (Loose leaven)	18	12.2	0.85	77.2	0.16	21.8	12.3	0.96	86.7	0.10	13.5
	IV (Loose leaven prod.)	18	12.5	0.85	75.4	0.17	23.2	12.3	0.96	87.7	0.10	13.5
	V (Sour)	3.5	7.9	0.53	74.7	0.11	23.4	8.0	0.61	84.7	0.08	16.7
	VI (Dough)	0.5	7.3	0.48	73.4	0.10	22.6	7.4	0.57	85.6	0.06	13.5
Production cycle	I (Loose leaven)	18	12.9	0.84	72.4	0.21	27.1	13.1	0.99	83.1	0.13	16.9
	II (Sour)	3.5	8.7	0.57	73.5	0.13	25.8	8.8	0.65	83.0	0.09	17.1
	III (Dough)	0.5	8.2	0.55	74.2	0.12	25.0	8.2	0.61	82.8	0.08	16.8

higher than in bread produced from spontaneously fermented starters. The penetrometric measurements of compressibility and relaxation of crumb (Table 4) pointed out that bread manufactured from starters with the participation of pure cultures was characterized 60 h after the end of baking, by identical parameters as bread from the traditional production after 24 h.

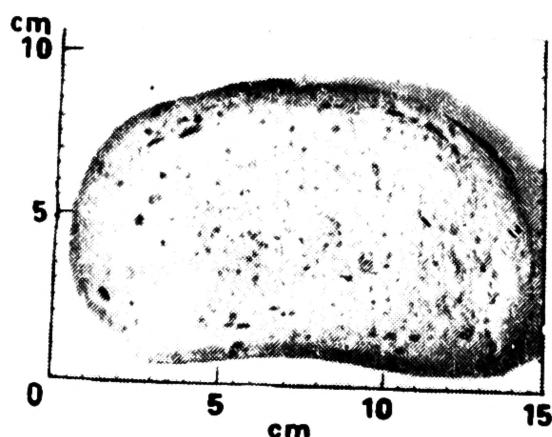


Photo 1. Bread obtained in the third day of the production cycle from spontaneously fermenting starters

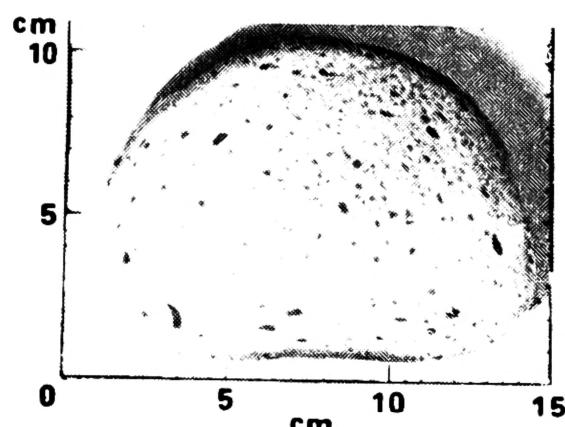


Photo 2. Bread obtained in the third day of the production cycle from starters fermented with *L. sanfrancisco* and *S. cerevisiae*

DISCUSSION

The presented results of the experiments have shown that in the industrial bread starters yeasts significantly prevailed (order of $10^3\text{-}10^4$) over lactic acid bacteria. Such state of microflora should be considered as unfavourable for a correct ripening of the environment, because as shown by the experiments of Ticha [40] and Spicher et al [29], in microbiologically good starters an dough, the number of lactobacilli should be from few to several score times higher than the number of yeast cells. In order to obtain the latter state especially in conditions of the industrial manufacture of bread — it is necessary to enrich the starter microflora with active cultures of lactic bacteria. It seems to result from the low physiological activity and weak tolerance to acidity of the environment of natural strains of *Lactobacillus* [28, 42, 45], as well as it may be connected with an often considerable addition of pressed yeasts. As a consequence, the symbiotic coexistence of both physiological groups is upset in favour of a state of competition leading to the dominance of yeasts [45]. Literature reports on the intensification of fermentation of bread starters present various forms of vaccines and the methods of their dosing. Wutzel [46] suggests a continuous method of feeding the fermentation containers with a paste of homo- and heterofermentative lactic bacteria.

T a b l e 2. Parameters of growth of acidifying bacteria and yeasts in loose bakery leavens in spontaneous processes and in a process with the participation of associated populations of *Lactobacillus sanfrancisco* and *Saccharomyces cerevisiae* A-1

Day of the cycle	Spontaneous fermentation						Fermentation with the share of pure cultures of <i>L. sanfrancisco</i> and <i>S. cerevisiae</i>						
	lag phase		μ max		generation period		lag phase		μ max		generation period		
	(h)	(h ⁻¹)	(h)	(h ⁻¹)	(h)	(h ⁻¹)	(h)	(h ⁻¹)	(h)	(h ⁻¹)	(h)		
Loose leavens introduced into 50-hour preliminary cycle	5.0	0.144	4.81	4.5	0.170	4.07	4.5	0.192	3.61	4.0	0.178	3.98	
	5.0	0.150	4.62	4.5	0.180	3.85	4.0	0.187	3.69	4.0	0.187	3.69	
Loose leavens production in 5-day cycle of bread production	1	5.5	0.132	5.25	5.0	0.169	4.10	4.5	0.150	4.62	4.0	0.157	4.41
	3	5.0	0.144	4.81	5.0	0.180	3.85	4.0	0.167	4.15	3.5	0.165	4.20
	5	5.0	0.144	4.81	5.0	0.180	3.85	4.0	0.167	4.15	4.0	0.161	4.33

Table 3. Technical evaluation of „praski” bread manufactured with spontaneously fermenting starters and starters with a share of associated populations of *Lactobacillus sanfrancisco* and *Saccharomyces cerevisiae* A-1

Day of cycle	Moisture (%)		Acidity ml 0.1N NaOH / 0.01 kg		Volume (cm ³ /0.1 kg)	
	bread from spontaneously fermenting starters	bread from starters fermented with the share of pure cultures	bread from spontaneously fermenting starters	bread from starters fermented with the share of pure cultures	bread from spontaneously fermenting starters	bread from starters fermented with the share of pure cultures
1	45.6	46.7	4.6	4.3	306	321
2	45.2	46.4	4.9	4.5	309	330
3	45.9	46.9	4.8	4.8	313	335
4	45.5	46.6	4.9	4.9	320	330
5	45.4	46.7	4.9	5.0	315	330

Table 4. Compressibility and relaxation of bread crumb of „praski” bread manufactured from spontaneously fermenting starters and from these with a share of associated populations of *Lactobacillus sanfrancisco* and *Saccharomyces cerevisiae* A-1

Time from the moment of baking (h)	Compressibility (°)*				Relaxation (°)*	
	crumb of bread with spontaneously fermented starters		crumb of bread with starters fermented with the share of pure cultures		crumb of bread with spontaneously fermented starters	crumb of bread with starters fermented with the share of pure cultures
	60 s	120 s	60 s	120 s	60 s	60 s
3	200	220	210	225	58	47
18	169	188	206	215	72	53
24	153	157	195	210	94	70
48	127	130	168	181	117	90
60	110	120	152	160	128	95
72	85	93	136	142	158	125

*¹ = 0.1 mm głębokość zanurzenia bolca probierczego

A similar idea is found in other American patents [6, 17]. In the western markets and in the United States, preparations of so-called “starter cultures” in the form of lyophilized or frozen concentrates of pure cultures are available [35].

Soviet experiments [11, 12, 13] and earlier studies of Sugihara et al [35] point to a good efficiency of the simplest inoculum in the form of 24-hour monocultures or of mixed populations of lactobacilli. Referring to these conceptions, the associated population of *L. sanfran-*

cisco and *S. cerevisiae* A-1 was utilized in the industrial experiments. It may appear controversial that the above mentioned composition of pure cultures was selected in the presence of a distinct predominance of yeasts in spontaneously fermenting starters. The choice of strains was based on the results of studies [44, 45] which confirmed the mutualistic character of coexistence of *L. sanfrancisco* and *S. cerevisiae* A-1 and expressed, among other things, by the mutual stimulation of growth and physiological activity.

The harmonious development of cultures introduced to the environment, visible in the equivalent quantitative relationships was maintained during the whole-week cycle of fermentation. Similar values of the growth parameters of both populations were also preserved which pointed to the leading role of *L. sanfrancisco* and *S. cerevisiae* A-1 in the ripening of starters. Fermentation directed by the share of the associated population was reflected in a 15% increased concentration of lactic acid Rohrlich [20] and Buskens [3] recognize the analogical share of lactic acid (80-85%) in the total group of acids as the optimal one for the rheological properties of dough and for the future organoleptic properties of bread. The obtained bread seems to confirm the suggestions of the authors. In standard evaluations, it distinguished itself in comparison with bread manufactured with spontaneously fermented starters. The penetrometric measurements of crumb confirm its very good quality and make it possible to suppose that bread obtained from starters fermented with the participation of pure cultures will preserve for a longer time its consumption freshness [19]. The obtained knowledge of the possibility of limiting (at least by 25%) of the recipe — in traditional technology — addition of pressed yeasts is a very important and economically measurable effect of fermentation directed by the participation of associated population of *L. sanfrancisco* and *S. cerevisiae* A-1.

CONCLUSIONS

1. Enrichment of microflora of industrial bread starters with the associated population of *L. sanfrancisco* and *S. cerevisiae* has contributed to a harmonious development and equivalent quantitative relationships of both physiological groups active in the medium.
2. In starters ripening with the participation of pure cultures, the concentration of lactic acid was by approx. 15% higher than in the spontaneously fermenting mediums.
3. Bread manufactured from starters fermented with the share of associated population of *L. sanfrancisco* and *S. cerevisiae* obtained the maximal assessment according to the standard rules.

4. The use of associated cultures of *L. plantarum* and *S. cerevisiae* in the conditions of industrial bread production could prolongate the consumption freshness of the product.

5. Technology of controlled fermentation of bread starters would make it possible to achieve considerable savings (about 25%) of pressed yeasts.

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SKOJARZONE KULTURY BAKTERII MLEKOWYCH I DROŻDŻY W PRZEMYSŁOWEJ PRODUKCJI CHLEBA

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Streszczenie

Skojarzone kultury *Lactobacillus sanfrancisco* i *Saccharomyces cerevisiae* wykorzystano w badaniach nad zidentyfikowaniem i ukierunkowaniem fermentacji przemysłowych zakwasów chlebowych. Inoculum w postaci 24 h hodowli skojarzonej tych szczepów dodawano w ilości stanowiącej 10% objętości fazy inicjującej tygodniowy cykl fermentacji zakwasów. W zakwasach fermentowanych z udziałem czystych kultur obserwowało się harmonijny rozwój obu czynnych grup drobnoustrojów. Z 10³-krotnej przewagi drożdży nad zdolnymi do wzrostu bakteriami mlekovymi stwierdzanej w zakwasach spontanicznie fermentujących następowało przesunięcie tej relacji do układu równoważnego i stan taki utrzymywał się w całotygodniowym cyklu odnawiania zakwasów fermentowanych z udziałem skojarzonej populacji *Lactobacillus sanfrancisco* i *Saccharomyces cerevisiae* (rys. 1-4). Zachowały się bliskie wartości parametrów wzrostu bakterii i drożdży (tab. 2), a czas fazy adaptacyjnej

uległy skróceniu dla obu populacji średnio o 45 min. Zakwasy fermentowane z udziałem czystych kultur charakteryzowały się poziomem kwasowości ogólnej identycznym jak próby z konwencjonalnej linii produkcji — zgodnym z wymaganiami normatywnymi (tab. 1). Znaleziono natomiast istotne różnice w składzie ilościowym głównych jej składowych: kwasu mleковego i kwasów lotnych. W zakwasach o kierowanej fermentacji kwas mlekowski stanowił ok. 85% kwasowości ogólnej, w próbach spontanicznie dojrzewających nie przekraczał 76% jej wartości (tab. 1). Chleb uzyskany z zakwasów, których fermentacja prowadzona była z udziałem czystych kultur cechował się znacznie korzystniejszymi walorami organoleptycznymi i parametrami technicznymi (o 6-10% większa objętość o 1,2% wyższa wilgotność) od wyprodukowanego wg konwencjonalnej technologii (tab. 3, 4, fot. 1, 2).