



THE EFFECT OF APPLICATION OF CHOSEN *LACTOBACILLI* STRAINS AS STARTER CULTURES ON RYE BREAD QUALITY

A. Ostasiewicz, A. Ceglińska, K. Bartochowska

*Department of Food Technology, Division of Cereal Technology,
Warsaw University of Life Science, Poland*

ABSTRACT

The research dealt with the evaluation of the influence of selected lactic acid bacteria belong to *Lactobacillus* genus on rye bread quality. The 720 type rye flour served for preparing the leaven with 300% efficiency. Lactic acid bacteria *Lactobacillus plantarum*, *Lactobacillus brevis*, and *Lactobacillus sanfranciscensis* were added into the leaven at the various amounts of 1, 2, 3 cm³ and then they were subject to fermentation for 24 and 48 h. The control sample (0) was composed of spontaneously fermenting leaven. Sourdough and subsequently dough was made of the leaven. Lactic acid bacteria, acetic acid bacteria, and yeast counts were determined in the dough. Produced breads were subject to sensory assessment by means of evaluating the following physicochemical features: 100 g bread volume, acidity and hardness of bread crumb, as well as baking loss was calculated. Enriching the sourdough with starter cultures affected the increase of lactic and acetic bacteria as well as yeast counts in the dough made using sourdough fermenting for 24 hours, regardless of the quantity and type of a culture applied. The largest bread volume recalculated onto 100 g was achieved when *Lactobacillus plantarum* NCAIMB.01149 strain was added into shorter-fermenting sourdough as well as after application of *Lactobacillus brevis* Lb2 strain for longer-fermenting sourdough. Compared to the control, those breads had also lower baking loss. The increase of bread crumb acidity was mostly influenced on by the addition of *Lactobacillus brevis* Lb2 and *Lactobacillus sanfranciscensis* Lb9 strains, after sourdough fermentation lasting both 24 and 48 hours. Breads containing these cultures achieved also the largest sum of scores in sensory assessment testing. Applied bacterial strains had no remarkable effects on bread crumb hardness. Worse crumb hardness was shown by breads produced from short-fermenting sourdough.

Key words: sourdough, lactic acid bacteria, starter cultures, rye bread quality

INTRODUCTION

The rye bread production resulting from a spontaneous fermentation of a sourdough has been known for thousands years [5]. Rye dough fermentation process consists of many stages that often have a common name "sourdough".

Therefore, the sourdough is the intermediate product containing active microorganisms, due to which the fermentation can occur [22]. Assuring the appropriate conditions to reproduce microflora beneficial for bread production is a prerequisite at every step of fermentation process, i.e. duration and temperature of fermentation, phase efficiency, and flour properties. The microflora is composed of symbiotic set of lactic acid fermentation bacteria and yeasts [5, 7, 9, 23]. Replacing the natural microorganisms with starter cultures makes fermentation reproducibility and stability reliable, because starter cultures are lactic acid bacteria and/or yeast populations selected from natural sourdough and having strict quantitative and qualitative composition and properties. There are following types of starter cultures: monocultures of homofermentative and heterofermentative lactic acid bacteria, most frequently from *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Weissella* genera, as well as conjugated lactic acid bacteria and yeast cultures, mainly of *Saccharomyces* genus [3, 10, 22, 38]. Presence of lactic acid bacteria is beneficial for technology and nutrition, as well as it influences on the bread quality [1, 2, 23, 35]. Acids produced by lactic acid bacteria (acetic, lactic, hydroxyacetic, formic, and pyruvic) along with other metabolic products (amino acids) affect the improved rheological features of a dough, hence it is more plastic, can be broken into parts more readily, and shows better oven spring feature. Furthermore, those products influence on better bread volume, texture, and flavor; they also delay the mould and bacterial spoilage processes [22, 36].

The present study aims was to evaluate the influence of chosen species of lactic acid fermentation bacteria from *Lactobacillus* genus (*Lb. plantarum*, *Lb. brevis*, and *Lb. sanfranciscensis*) on microflora in rye dough and rye bread quality.

MATERIALS AND METHODS

The 720 type rye flour purchased in one of mills (Polish Mills), instant dry yeast purchased in a local supermarket, and strains of lactic acid bacteria (LAB) applied as starter cultures for sourdough fermentation, such as: *Lactobacillus plantarum* NCAIMB.01149, *Lactobacillus brevis* Lb2, and *Lactobacillus sanfranciscensis* Lb9 originating from Division of Milk Biotechnology of Warsaw University of Life Science, were used to prepare dough for baking breads.

Physicochemical properties of rye flour. The assessment of physicochemical traits of 720 type rye flour included moisture content [12], titratable acidity [27], total protein content [28], falling number [33], and flour amyolytic properties [34], applying techniques and methods included in Polish Norms.

Preparation of LAB cultures. Under septic conditions, aliquots of 0.1 g of the following starter cultures were added into tubes containing 10 ml of MRS (Merck, Germany) broth: *Lactobacillus plantarum* NCAIMB.01149, *Lactobacillus brevis* Lb2, and *Lactobacillus sanfranciscensis* Lb9. Samples were stirred to dissolve solids and placed at the temperature optimum for a given inoculum (30°C) for 24 hours. After that period, cultures were stirred and 1 ml of sterile suspension was re-inoculated into tube filled with 9 ml of MRS broth. The biomass was concentrated 15 times up to 10 ml of volume in centrifuge MPW-350R at 4000 rpm for 6 minutes. Such prepared cultures were added into the leaven at the amounts of 1, 2, and 3 ml.

Bread baking procedure. The dough for baking was made in three steps (leaven – I, sourdough – II, and dough – III). Leaven was prepared using 100 g of rye flour and 200 g tap water. Lactic acid bacteria cultures were added into leavens at the amounts of 1, 2, and 3 ml. The control (0) was composed of spontaneously fermented, no bacteria-added leaven. The sourdough was made of fermented leavens with flour and water addition according to the recipe. After the sourdough fermentation complete (3 hours), the dough was prepared by mixing it with flour, water, dry yeasts, and salt (1.5% and 2.5% respectively referring to flour weight) and mixed for 4 minutes achieving dough with 170% efficiency. The dough fermented for another 30 minutes at 30°C, then it was broken into parts of 350 g each and transferred into molds. Such divided dough fragments grew within fermentation chamber for 1 h at 30°C and 75% moisture content, and subsequently bread was baked in electric oven (Sveba Dahlen, Sweden) at 240°C for 35 minutes.

Microbiological analysis. Microbiological dilutions were made by taking 1 g of dough and placing into sterile foil bags. Then the sample was immersed in 9 ml of peptone solution (BTL, Poland) and homogenized for 60 seconds in homogenizer (Seward Stomacher 80, USA). Thus, 10^{-1} dilution was achieved. In order to get other dilutions, aliquot of 1 ml working dilution was transferred into sterile tubes containing 9 ml of peptone solution. Each time, tubes were thoroughly shaken to achieve homogenous solution [31].

Determination of LAB count in a dough was made by means of droplet method. Petri plates with MRS–Agar substrate (Merck, Germany) were divided into quarters and 20 μ l of the dilution was poured out onto each one. Plates were incubated in anaerobiosis at 30°C for 3 days. Determination of yeast and mould cells count was made by means of the deep-seated inoculation method. Volume of 1 ml of each dilution was transferred onto Petri plates and flooded with YGC–Agar substrate (Merck, Germany). Plates were then incubated at 25°C for 5 days. The count of acetic acid bacteria was determined applying deep-seated method. Aliquots of 1 ml of each dilution were added to a substrate containing yeast extract and ethanol. Petri plates were incubated at 30°C for 3 days. After incubation,

grown colonies counts were recalculated onto the logarithm of bacterial and yeast cell colony forming units ($\log_{10}\text{cfu g}^{-1}$). All determinations were performed in two parallel replicates.

Physicochemical analysis of breads. The evaluation of physicochemical properties of produced bread was carried out 20 hours after the baking. The bread baking loss value was calculated as a difference between weight of the dough part before baking and bread weight when taken out of the oven. Bread volume was calculated with a help of loose materials (rapeseed grains) with subsequent recalculation onto the volume of 100 g baked bread. Bread crumb acidity as total titratable acidity (TTA) was determined by titration with 0.1 N NaOH and expressed in terms of milliliters of NaOH. The crumb hardness was measured as a maximum press force (N) using texture analyzer TA.XT2 (Stable Micro Systems, UK) according to the manual [18]. Measurements were made in two replicates.

Organoleptic evaluation. The sensory assessment of bread at 20 h after baking was made by qualified group of persons. The external properties of bread (volume, shape, crust color and thickness) and internal properties (elasticity and porosity of the crumb), as well as taste and flavor were subject to evaluation. The sensory traits were ranked to classify produced breads to particular bakery quality groups [32].

Statistical analysis. Achieved results were statistically processed with a help of Statgraphics Plus 4.1 software. One-way ANOVA variance analysis was performed. Significant statistical differences between mean values depending on the effects of various factors, were verified applying Tukey HSD test.

RESULTS AND DISCUSSION

Analysis of physicochemical traits of 720 type rye flour revealed that it was characterized by quite good rheological properties. Table 1 presents results illustrating the quality of 720 type rye flour used in the experiments.

Table 1. Quality characteristics of the rye flour type 720, mean \pm S.D.

Properties	Mean \pm S.D.
Moisture content, %	13,3 \pm 0,08
Titratable acidity, acidity degree	3,51 \pm 0,01
Protein content, %	7,80 \pm 0,47
Falling number, s	202 \pm 0,58
Maximum viscosity of gelatinized slurry, UA	390 \pm 0,01

S.D. – standard deviation

Moisture content and acidity of examined flour were consistent with recommendations of Polish Norms [29], hence they were suitable to produce rye bread products in industrial bakeries. Protein content in studied flour was 7.80%, which was similar to the rye flour applied in experiments performed by Michalska et al. [21]. Despite of lower contents of proteins in rye rather than wheat flour, plays an important role in shaping the technological properties of dough and bakery. Usefulness of the rye flour for bread production is determined by properties of starch contained such as ability to gelatinization and susceptibility to amylolytic enzymes action. Amylolytic activity of examined flour was moderate, which was indicated by falling number value (202 s). Falling number apparatus measures the time it takes a plunger to move through the viscous gelatinized slurry. The longer it takes, the more viscous the slurry is and the less damaged starch present in the sample [36]. During sourdough baking, low values of the falling number are preferred, because high amylolytic activity ensures a strong and rapid start for fermentation and also improves a characteristic flavor [39]. Other researchers used rye flour type 720, which has lower value of the falling number (173 s) [24] or higher (302 s) [6]. Viscosity of gelatinized water suspension of the flour was 390 (UA), which can also confirm moderate amylolytic activity of studied rye flour.

Table 2 lists data on quantitative composition of microflora in the dough (lactic and acetic acid bacteria, yeasts) produced of the leaven fermenting for 24 and 48 h, and enriched with lactic acid bacteria at three different rates.

Table 2. The influence of the time of carrying out of leaven and quantity of starter cultures applied on sourdough microflora, mean \pm SD

Sample	Fermentation time [h]	Quantity of starter cultures [ml]	Count of microflora [\log_{10} cfu g ⁻¹]		
			LAB	Acetic bacteria	Yeast
A	24	1	8,72 \pm 0,09	8,47 \pm 0,12	7,60 \pm 0,14
		2	8,42 \pm 0,17	8,40 \pm 0,13	7,74 \pm 0,04
		3	8,09 \pm 0,29	8,41 \pm 0,04	7,66 \pm 0,05
	48	1	8,36 \pm 0,26	n.g.	7,65 \pm 0,01
		2	7,30 \pm 0,00	n.g.	7,54 \pm 0,10
		3	8,07 \pm 0,09	n.g.	7,50 \pm 0,07
B	24	1	8,72 \pm 0,34	8,46 \pm 0,04	7,72 \pm 0,01
		2	8,54 \pm 0,00	8,68 \pm 0,03	7,72 \pm 0,08
		3	8,53 \pm 0,18	8,64 \pm 0,03	7,75 \pm 0,03
	48	1	8,51 \pm 0,05	8,58 \pm 0,05	7,78 \pm 0,01
		2	8,93 \pm 0,07	8,60 \pm 0,01	7,88 \pm 0,01
		3	8,70 \pm 0,06	8,74 \pm 0,06	7,82 \pm 0,02
C	24	1	8,85 \pm 0,43	8,37 \pm 0,10	7,51 \pm 0,03
		2	9,15 \pm 0,31	8,80 \pm 0,14	7,74 \pm 0,01
		3	8,74 \pm 0,06	8,67 \pm 0,01	7,59 \pm 0,05
	48	1	8,67 \pm 0,10	8,53 \pm 0,21	7,74 \pm 0,02
		2	8,11 \pm 0,29	8,35 \pm 0,07	7,74 \pm 0,06
		3	8,60 \pm 0,00	8,47 \pm 0,05	7,59 \pm 0,10
Control	24	0	7,89 \pm 0,18	8,03 \pm 0,03	7,41 \pm 0,05
	48	0	8,64 \pm 0,31	8,61 \pm 0,05	7,73 \pm 0,04

A – *Lb. plantarum* NCAIMB.01149

B – *Lb. brevis* Lb2

C – *Lb. sanfranciscensis* Lb9

n.g. – no growth detected

The LAB count in the dough amended with starter cultures and made using the leaven fermenting for 24 h amounted from 8.1 to 9.1 log cfu/g, which was higher than in control sample (7.9 log cfu/g). Number of these bacterial cells in the leaven fermenting for 48 h was more diverse and oscillated within 7.3 to 8.9 log cfu/g. Yeast cell count in all sorts of dough was similar: from 7.41 to 7.88 log cfu/g. Gül et al. [10] reported that LAB cells count in experimental dough types ranged from 5.28 to 9.66 log cfu/g, while yeast cell number from 6.33 to 9.96 log cfu/g. It was also observed that LAB cells count was by 1 or 2 logarithmic orders higher than yeast cells number. It is consistent with literature data indicating that properly soured leavens should contain the yeast to LAB ratio as 1:10 or 1:100 [3,11]. Dough prepared of the leaven fermenting for 24 h and enriched with *Lb. plantarum* NCAIMB.01149 bacteria contained more LAB, acetic acid, and yeast cells as compared to that functioning as a control. Number of yeast cells was lower in the dough amended with the same bacterial species, but after longer leaven fermentation (48 h) than in the control.

In the dough enriched with *Lb. plantarum* NCAIMB.01149 culture, the leaven fermentation duration (24 h or 48 h) had no influence on mould presence, because some *Lb. plantarum* strains produce antifungal agents such as phenyllactic, 4-hydroxy-phenyllactic acids, and two cyclic dipeptides (cyclo L-Leu-Pro and cyclo L-Phe-trans-4OH-L-Pro), that are efficient in inhibiting the mould development[4, 17].

Presence of acetic acid bacterial cells was not detected in the dough made of the leaven fermenting for 48 h and amended with *Lb. plantarum* NCAIMB.01149. Adding *Lb. plantarum* into the leaven may inhibit the acetic acid

bacteria development in a dough. Studies made by Katina et al. [15] reported that larger amounts of *Lb. plantarum* in a dough also effectively inhibited development of bacteria from *Bacillus* genus, especially *Bacillus subtilis*. Similar results were also achieved by Şimşek et al. [37]. They reported that the species addition had inhibitive action towards *Escherichia coli* and *Staphylococcus aureus* bacteria growth.

The LAB count in the dough with *Lb. brevis*Lb2 addition remained at comparable level (8.5–8.9 log cfu/g) both at different doses of the starter culture, and various durations of the leaven fermentation. The quantity of added bacterial cultures along with the leaven fermentation duration had no remarkable impact on yeast cells number in the dough with *Lb. brevis* Lb2 addition (7.7–7.9 log cfu/g).

Number of acetic acid bacteria also remained at similar levels (8.5–8.7 log cfu/g) regardless of the amount of added *Lb. brevis*Lb2 strain, nor the leaven fermentation duration.

Dough produced from the leaven fermenting for 24 h with *Lb. sanfranciscensis*Lb9 strain addition was characterized by lactic acid bacteria cells count from 8.9 to 9.2 log cfu/g. Number of LAB cells was more diversified and depended on the quantity of added starter culture in the dough made of the leaven fermenting for 24 h. The most intensive growth of LAB cells in the dough – in reference to the control – was observed when 2 ml of that culture was added, whereas the number of LAB count in the dough produced of the leaven fermenting for 48 h with *Lb. sanfranciscensis* Lb9 addition ranged from 8.1 to 8.7 log cfu/g. Moreover, *Lb. sanfranciscensis* bacteria presence in a leaven had stimulating effects on the increase of LAB count, while it prevented from the development of undesired microflora, e.g. sporulating bacteria such as *Bacillus subtilis* [8].

The yeast cells count remained at very similar level (7.51–7.74 log cfu/g) regardless of the quantity of added *Lb. sanfranciscensis* Lb9 culture as well as the leaven fermentation duration; however, it was slightly higher as compared to the number of yeasts in the control. Number of acetic acid bacteria cells in the dough made of short-fermenting leaven and enriched with *Lb. sanfranciscensis* Lb9 culture was higher (8.37–8.80 log cfu/g) than in the control sample. The inverse dependence was observed in the dough made of the leaven fermenting for 48 h (8.35–8.53 log cfu/g). In that type of dough, the acetic acid bacteria count remained at comparable levels regardless of the amount of added *Lb. sanfranciscensis* Lb9.

Table 3 presents general bread quality determinants such as baking loss, volume of 100 g bread, as well as acidity and hardness of breadcrumb. The lowest baking loss comparing to the control (10.18%) characterized bread with *Lb. sanfranciscensis* Lb9 addition and made of the leaven fermenting for 24 h (9.62–9.92%). The lowest baking loss (7.94%) characterized bread with 2 ml addition of this starter culture after 48 h of leaven fermentation. Breads with addition of *Lb. plantarum* NCAIMB.01149 based on longer-fermenting leavens showed the highest baking loss. Except from the bread produced with addition of 2 ml *Lb. sanfranciscensis* Lb9 strain, longer duration of the leaven fermentation affected the increased bread baking loss, while the quantity and type of starter culture added had no major influence on bread baking loss.

Table 3. The effect of starter cultures applied and fermentation time on rye bread quality

Sample	Fermentation time [h]	Quantity of starter cultures [ml]	Baking loss [%]	Volume of 100 g of bread [cm ³]	Titration acidity [Acidity degree]	Hardness of bread crumb [N]
A	24	1	10,58 b ± 0,20	247,4 c ± 2,12	2,76 ab ± 0,24	9,20 cd ± 0,88
		2	9,66 ab ± 0,08	232,9 c ± 0,99	2,96 ab ± 0,12	5,11 ab ± 0,39
		3	9,54 ab ± 0,08	208,3 bc ± 0,92	3,12 ab ± 0,02	8,20 bcd ± 0,91
	48	1	11,82 b ± 0,59	159,1 a ± 1,77	2,83 a ± 0,15	7,31 bc ± 0,88
		2	11,78 b ± 0,14	137,5 a ± 2,69	2,86 a ± 0,08	6,26 ab ± 1,33
		3	12,92 b ± 0,06	126,3 a ± 1,13	2,63 a ± 0,06	7,32 bc ± 1,72
B	24	1	8,28 ab ± 0,25	146,1 a ± 0,42	3,12 ab ± 0,23	10,69 d ± 1,02
		2	10,28 b ± 0,51	153,3 a ± 1,63	3,27 b ± 0,00	9,16 cd ± 0,38
		3	9,92 ab ± 0,17	155,0 a ± 0,35	3,44 b ± 0,21	10,19 cd ± 0,2
	48	1	10,32 ab ± 0,11	254,3 c ± 0,21	3,47 b ± 0,23	9,77 cd ± 0,16
		2	10,18 ab ± 0,31	241,8 bc ± 2,55	3,57 bc ± 0,28	9,97 cd ± 0,21
		3	10,88 ab ± 0,11	237,1 ± bc 5,81	3,52 b ± 0,02	10,07 d ± 0,13
C	24	1	9,72 ab ± 0,31	136,8 a ± 6,08	2,91 ab ± 0,04	6,27 abc ± 0,71
		2	9,92 ab ± 0,23	128,9 a ± 3,89	3,03 ab ± 0,07	3,56 a ± 0,93
		3	9,62 ab ± 0,08	155,6 a ± 1,13	3,17 ab ± 0,03	7,57 bcd ± 0,11
	48	1	10,06 ab ± 0,14	140,6 a ± 2,55	3,86 bc ± 0,04	5,70 ab ± 1,12
		2	7,94 a ± 0,54	150,3 a ± 0,57	3,92 bc ± 0,06	4,66 ab ± 2,31
		3	10,52 ab ± 0,56	173,9 ab ± 2,33	4,13 c ± 0,08	4,19 a ± 1,74
Control	24	0	10,18 b ± 0,29	157,2 ab ± 2,78	2,03 a ± 0,08	8,13 bcd ± 0,82
	48	0	10,99 ab ± 0,53	203,8 abc ± 2,62	3,42 b ± 0,04	6,36 ab ± 0,88

A – *Lb. plantarum* NCAIMB.01149

B – *Lb. brevis* Lb2

C – *Lb. sanfranciscensis* Lb9

The same letters (a–d) in columns mean groups not significantly different at p=0.05

The largest volume of 100 g was revealed by breads made of the leaven fermenting for 48 h with *Lb. brevis* Lb2 addition (237–254 cm³) as well as those produced of leavens fermenting for 24 h and containing *Lb. plantarum* NCAIMB.01149 (208.3–247.4 cm³). In both cases, the largest volume was found for breads with 1 ml starter cultures addition. Along with the increase of added starter culture, bread volume decreased. Other loaves of bread were characterized by much lower volumes than the control samples (157.2 and 203.8 cm³), which cannot confirm the results achieved by Plessas et al. [26], who reported that all adding the starter cultures positively affected the bread volume as compared to that made with no starters addition, the volume of which was lower.

The crumb acidity of the bread enriched with the starter cultures was higher than that of the control, except from bread containing *Lb. plantarum* NCAIMB.01149 culture after 48 hours of sourdough fermentation (2.63–2.86° of acidity). Breads with *Lb. sanfranciscensis* Lb9 addition and made of longer-fermenting leavens were characterized by the highest acidity of the crumb. These breads acidity increased along with the increase of the starter culture dose. These results are confirmed by research performed by Plessas et al. [26], who reported that bread enriched with starter cultures was characterized by higher acidity as compared to that produced as a result of spontaneous fermentation, which was due to the larger amounts of lactic and acetic acids released into a sourdough [20, 25].

Hardness of the bread crumb was very diverse. Bread produced of the leaven fermenting for 24 h with addition of 2 ml *Lb. sanfranciscensis* Lb9 strain was characterized by the lowest hardness (3.56 N). Crumb hardness of studied breads decreased along with the increase of starter culture dose. For breads containing 1 and 3 ml of starter cultures, as well as in the control, the crumb hardness decreased along with the leaven fermentation duration, as the same to

the results achieved by other researchers [13, 14,16], who reported that longer duration of the sourdough fermentation affected the lower hardness of the bread crumb. On the other hand it is known, that while crumb hardness of bread is decreasing, staling of bread is delayed that mean shelf life of bread increases [10].

Table 4 presents results from the sensory traits assessment ranking. The assessment consists in illustrating the intensity of every quality trait using numerical values according to accepted scoring system, which expresses the total quality of assessed bread (maximum possible total score is 32) [32].

Table 4. Organoleptic assessment of bread

Sample	Fermentation time [h]	Quantity of starter cultures [ml]	The sum of points	Quality level
A	24	1	24	II
		2	23	II
		3	18	III
	48	1	29	I
		2	26	II
		3	25	II
B	24	1	30	I
		2	30	I
		3	27	II
	48	1	30	I
		2	30	I
		3	28	I
C	24	1	26	II
		2	28	I
		3	28	I
	48	1	28	I
		2	27	II
		3	29	I
Control	24	0	24	II
	48	0	29	I

A – *Lb. plantarum* NCAIMB.01149

B – *Lb. brevis* Lb2

C – *Lb. sanfranciscensis* Lb9

The highest scores were granted to breads with *Lb. brevis* Lb2 strain and made of the leavens fermenting for 48 h. These breads were classified to the 1st quality level of bread. Breads with *Lb. sanfranciscensis* Lb9 culture addition also gained high scores during sensory assessment. The lowest scores were granted to bread containing 3 ml *Lb. plantarum* NCAIMB.01149 and produced after 24 h of leaven fermentation (18 points). Studies made by Martinez-Anaya et al.[19] revealed that *Lb. plantarum* and *Lb. brevis* had positive impact on sensory features of bread products. Moreover, the larger quantity of a starter culture added, the better tastiness and flavor of a bread [26], which was achieved in the present research for the bread with *Lb. sanfranciscensis* Lb9 culture addition. Bread with no starter cultures added (control sample) was also characterized by good general quality, which was confirmed by the 1st quality level of that bread.

CONCLUSIONS

As comparing to the control, addition of starter cultures influenced on enhancement in lactic and acetic bacteria as well as yeast counts, regardless of the amount and type of applied culture, in dough achieved after short sourdough fermentation (24 hours). Unlike in a dough made from longer-fermenting sourdough (48 hours), despite of *Lb. plantarum* NCAIMB.01149 and *Lb. sanfranciscensis* Lb9 cultures were used, number of lactic bacteria cells was lower than in the control. In this case, acetic bacteria population also decreased. The yeast cell count in dough with *Lb. brevis* Lb2 and *Lb. sanfranciscensis* Lb9 strains addition was slightly higher than in the control. Final bread volume was mostly affected by adding 1 cm³ of *Lb. plantarum* NCAIMB.01149 strain into short-fermenting sourdough (24 h) as well as *Lb. brevis* Lb2 to sourdough fermenting for 48 h, because a considerable increase as compared to the control was recorded. Addition of *Lb. brevis* Lb2 and *Lb. sanfranciscensis* Lb9 cultures had the greatest impact on the increase of bread crumb acidity, hence on higher scores during the bread sensory assessment. Achieved results allowed for concluding that addition of lactic acid bacteria in a form of starter cultures into the rye bread production had some influences on the quantitative microflora composition in a dough. Both the type and amount of added starter culture, as well as the leaven fermentation duration had effects on physicochemical and sensory features of a final bread. Summarizing, applying starter cultures in a form of lactic acid monoculture affects the improvement of rye bread quality.

Acknowledgements

The authors acknowledge their financial support of the study from the Ministry of Science and Higher Education as a part of a project (Project No. NN312 122539). We thank to Division of Milk Biotechnology for providing starter cultures to the research.

REFERENCES

1. Bamforth C.W., 2005. Food fermentation and micro-organisms. Blackwell Sci., (pp. 172–181).
2. Blandinob A., Al-Aseeria M.E., Pandiella SS., Canterob D.,Webba C., 2003. Cereal-based fermented foods and beverages. Food Res. Int., 36, 527–543.
3. Corsetti A., Settanni L., 2007. Lactobacilli in sourdough fermentation. Food Res. Int., 40, 539-558.
4. Dal Bello F., Clarke C.I., Ryan L.A.M., Ulmer H., Schober T.J., Ström K., SjögrenJ., van Sinderen D., Schnürer J., Arendt E.K., 2007. Improvement of the quality and shelf life of wheat bread by fermentation with the antifungal strain *Lactobacillus plantarum* FST 1.7. J. Cereal Sci., 45, 309-318.
5. Decock P., Cappelle S., 2005. Bread technology and sourdough technology. Trends Food Sci. Technol., 16, 113–120.
6. Denli E., Ercan R., 2001. Effect of added pentosans isolated from wheat and rye grain on some properties of bread. European Food Res. Technol., 212, 374–376.
7. Gobbetti M., 1998. The sourdough microflora: Interactions of lactic acid bacteria and yeasts. Trends Food Sci. Technol., 9, 267–274.
8. Gobbetti M., Corsetti A., 1997. *Lactobacillus sanfrancisco* a key sourdough lactic acid bacterium: review. Food Microbiol., 14, 175–187.
9. Gobbetti M., De Angelis M., Corsetti A., Di Cagno R., 2005. Biochemistry and physiology of sourdough lactic acid bacteria. Trends Food Sci. Technol., 16, 57–59.
10. Gül H., Özçelik S., Sağdıç O., Certel M., 2005. Sourdough bread production with lactobacilli and *S. cerevisiae* isolated from sourdoughs. Process Biochem., 40, 691–697.
11. Iacumin L., Cecchin, F., Manzano M., Osualdini M., Boscolo D., Orlic S.,Comi G., 2009. Description of the microflora of sourdoughs by culture-dependent and culture-independent methods. Food Microbiol., 26, 128–135.
12. ISO 711:1985. Cereals and cereal products. Determination of moisture content (basic reference method).
13. Katina K., Arendt E., Liukkonen K.H., Autio K., Flander L.,Poutanen K., 2005. Potential of sourdough for healthier cereal products. Trends Food Sci. Technol., 16, (3), 104–112.
14. Katina, K., Heinio R.L., Autio K.,Poutanen K., 2006. Optimization of sourdough process for improved sensory profile and texture of wheat bread. LWT-Food Sci.Technol., 39, (10),1189–1202.
15. Katina K., Sauri M., Alakomi H-L., Matilla-Sandholm T., 2002. Potential of lactic acid bacteria to inhibit rope spoilage in wheat sourdough bread. Lebensmittel-Wissenschaft und Technologie, 35, 38–45.
16. Kim Y., Huang W., Zhu H., Rayas-Duarte P., 2009. Spontaneous sourdough processing of Chinese Northern-style steamed breads and their volatile compounds. Food Chem., 114, 685–692.
17. Lavermicocca P., Valerino F., Visconti A., 2003. Antifungal activity of phenyllactic against moulds isolated from bakery products. App. Environ.Microbiol., 69, 634–640.
18. Manual. Texture Analyzer TA.XT2 (Stable Micro Systems) 1997.
19. Martinez-Anaya M. A., Pitarch B., Barber B.C., 1993. Biochemical characteristics and bread making performance of freeze-dried wheat sour dough starters. ZeitschriftfürLebensmittelUntersuchung und Forschung, 196, (4), 360–365.
20. Meignen B., Onno B., Gélinas P., Infantes M., Guilois S., Cahagnier B., 2001. Optimization of sourdough fermentation with *Lactobacillus brevis* and baker's yeast. Food Microbiol., 18, 239–245.

21. Michalska A., Amigo-Benavent M., Zieliński H., Dolores del Castillo M., 2008. Effect of bread making on formation of Maillard reaction products contributing to the overall antioxidant activity of rye bread. *J.Cereal Sci.*, 48, 123–132.
22. Mozzi F., Raya R. R., Vignolo G.M., 2010. Biotechnology of Lactic Acid Bacteria. Novel applications. In G. Font de Valdez, C.L. Gerez, M.I. Toriono, G. Rollán (Eds.), *New trends in cereal-based products using Lactic Acid Bacteria* (pp.273–287), Ames: Wiley-Blackwell, Inc.
23. Neysens P., De Vuyst L., 2005. Kinetics and modeling of sourdough lactic acid bacteria. *Trends Food Sci.Technol.*, 16, 95–103.
24. Ostasiewicz A., Ceglińska A., Skowronek S., 2008. Wpływ warunków prowadzenia zakwasu na jakość chleba żytniego. *Żywność. Nauka. Technologia. Jakość.* (The influence of condition of carrying out of sourdough on the rye bread quality. *Food. Science. Technology. Quality.*), 5, (60), 34–42.
25. Paramithiotis S., Gioulatos S., Tsakalidou E., Kalantzopoulos G., 2006. Interactions between *Saccharomyces cerevisiae* and lactic acid bacteria in sourdoughs. *Process Biochem.*, 41, 2429–2433.
26. Plessas S., Fisher A., Koureta K., Psarianos C., Nigam P., Koutinas A., 2008. Application of *Kluyveromyces marxianus*, *Lactobacillus delbrueckii* ssp. *bulgaricus* and *L. helveticus* for sourdough bread making. *Food Chem.*, 106, 985–990.
27. PN-60/A-74007. Cereal products. Acidity determination.
28. PN-75/A-04018. Food products. Determination of nitrogen by means of Kjeldahl method and recalculation onto protein content.
29. PN-86/A-74034. Cereal products. Rye flour.
30. PN-93/A-86034/02. Milk and milk products. Microbiology analysis. Methods of research.
31. PN-93/A-86034/03. Milk and milk products. Microbiology analysis. Preparing samples and dilutions.
32. PN-A-74108:1996. Bakery products. Methods of research.
33. PN-ISO 3093/AK:1982. Cereals. Determination of falling number.
34. PN-ISO 7973:2001. Cereals and milled cereal products. Determination of viscosity of flour. Method using an amylograph.
35. Salminen S., Wright A., Ouwehand A., 2004. *Lactic Acid Bacteria: Microbiological and functional aspects.* (3rd ed.). New York: Marcel Dekker, Inc.
36. Serna-Saldivar S.O., 2010. Cereal grains. Properties, processing, and nutritional attributes. Boca Raton: CRC Press, (Chapter 10).
37. Şimşek Ö., Çon A. H., Tulumoğlu Ş., 2006. Isolating lactic starter cultures with antimicrobial activity for sourdough processes. *Food Control*, 17, 263–270.
38. Temmerman R., Huys G., Swins J., 2004. Identification of lactic acid bacteria: culture-dependent and culture-independent methods. *Trends Food Sci.Technol.*, 15, 348–359.
39. Zieliński H., Michalska A., Ceglińska A., Lamparski G., 2008. Antioxidant properties and sensory quality of traditional rye bread as affected by the incorporation of flour with different extraction rates in the formulation. *Eur. Food Res. Technol.*, 226, 671–680.

Anna Ostasiewicz

Department of Food Technology, Division of Cereal Technology, Warsaw University of Life Science, Poland
Nowoursynowska 159 c, 02-787 Warsaw,
Phone: +48 (0) 22 5937542, Fax: +48 (0) 22 5937539,
e-mail: anna_ostasiewicz@sggw.pl

Alicja Ceglińska

Department of Food Technology, Division of Cereal Technology, Warsaw University of Life Science, Poland
Nowoursynowska 159 c, 02-787 Warsaw,
Phone: +48 (0) 22 5937542, Fax: +48 (0) 22 5937539,
e-mail: alicja_ceglinska@sggw.pl

Katarzyna Bartochowska

Department of Food Technology, Division of Cereal Technology, Warsaw University of Life Science, Poland
Nowoursynowska 159 c, 02-787 Warsaw
