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ATTEMPT TO OBTAIN BEVERAGE CONTAINING VIABLE LACTIC ACID BACTERIA AND ESTIMATION OF THEIR SURVIVAL ABILITY AT THE SELECTED TEMPERATURES

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Abstract. The aim of this work was to obtain "malt beverage" which includes viable cultures of lactic acid bacteria from *Lactobacillus plantarum* species and to estimate lifetime of these bacteria during 2-months of storing in temperature of 22°C and 6°C. After concluding storage period, a number of bacteria cells was in a range of 10^7-10^8 cfu/cm^3 that is at higher level than minimum numbers of lactic acid bacteria needed for probiotical products. A decrease of pH value from 3.97 to 3.46 and 3.28 was observed in relation to storage temperature and an increase of total acidity from 0.14 to 0.27 and 0.60 g/100 cm^3 of a product.
After storage period /and during it/ a good sensor quality of "malt beverage" was reported.

Key words: lactic acid bacteria, probiotic, ability of survival, storage, kvass, "malt beverage"

INTRODUCTION

Generally by using probiotic term we describe biological specimens, which include single or mixed cultures of microorganisms positively influencing a condition of health of people by maintaining or restoring microbiological equilibrium in a digestive tract, and sometimes by other reactions [Huis in't Veld and Havenaar 1991, Usajewicz 1999]. Many research results confirm pro-healthy reactions of these products on human organism which depend on: prophylactic and therapeutic measures in diarrhoea and food poisoning, alleviation of wide effect influence of antibiotic therapy, alleviation of lactose intolerance, anticarcinogenic action, stimulation of immunity system, decreasing a level of cholesterol in blood, prevention of osteoporosis [Bakterie fermentacji... 1998, Marteau and Rambaud 1993, Prost 1999, Sanders 1999, Usajewicz 1999].

In the research that has to confirm probiotic characteristics of the tested strain we can find three groups of characteristics:

– general requirements, i.e. origin, safety, resistance to low pH, bile acids and gastric juices,

– functional aspects, i.e. ability of adherence to intestinal epithelium, antagonism to pathogens,

– technological requirements this means survival ability, activity in technological processes [Probiotyki 2002].

Probiotic specimens include viable cells of microorganisms, generally lactic acid bacteria of genera *Lactobacillus, Bifidobacterium and Streptococcus,* and also yeast *Saccharomyces cerevisiae, Torulopsis* [Grzybowski et al. 1997, O'Sullivan et al. 1992].

Most available food probiotic products on the market are fermented dairy beverages which include live cultures of lactic acid bacteria. Milk diet normally is not a base of food for adult, and what is more some people suffer from lactose intolerance or allergy to milk protein. In most cases these are vegetable products therefore technologists try to enlarge products range which include lactic acid bacteria with such products as salads, juices or other obtained from vegetable raw materials [Lee and Salminen 1995, Łaniewska-Moroz et al. 1996]. Especially, we have to consider selected species of *Lactobacillus* genus, which live in vegetable habitat, they are used in souring of vegetable products [Daeschel and Nes 1994, Krawczyk 1987, Łaniewska-Moroz and Rocznia-kowa 1993]. Bacteria belonging to *Lactobacillus plantarum* have good technological and health properties (among them there is resistance to low pH and high acidity, production ability of bacteriocins, safety of use, and some strains were isolated from the human oral cavity and intestinal tract) [Daeschel and Nes 1994, Molin 1998].

The product, which is of vegetable origin and provides a consumer with lactic acid bacteria, seems to be kvass or a beverage of similar type. Kvass is popular in the countries of the former Soviet Union. Kvass is produced from wort or its concentrate, obtained from special rye-malt, barley-malt, rye-flour, sugar and other taste additions. This product undergoes previous limited alcoholic and lactic acid fermentation and includes viable lactic acid bacteria. Bacterial mixture composition used for inoculation is not given. It is sold from barrel trucks and its stability is of 2 to 3 days. Kvass sold in bottles is not produced by fermentation method and does not have lactic acid bacteria, but always contains citric acid and preservatives [Kovalski 1997].

In this work we tried to determine survival ability of lactic acid bacteria during beverage storing with characteristics similar to kvass, which is further on called "malt beverage". We wanted to answer if produced beverage with viable lactic acid bacteria may have considerably long (in industry scale) period of validity for consumption and such a number of bacteria that it is possible to fulfil condition for products indexed with "bio"prefix. We also searched for changes of chosen chemical components during the process. In experiments, we used "malt beverage" (not kvass) because of simplified technology of its preparation (for example from a concentrate of malt wort).

MATERIALS AND METHODS

In the research we used lactic acid bacteria:

– *Lactobacillus plantarum 44* strain from Department of Biotechnology and Food Microbiology, SGGW,

– *Lactobacillus plantarum 299 v* isolated from ProViva beverage (Skane Dairy – Sweden).

Experiment was carried out using "malt beverage wort" obtained by dissolution and mixing in ratio used in initial tests of the following components: consumption sugar, industrial malt concentrate (produced by Wytwórnia Ekstraktu Słodowego Wolsztyn), caramel, citric acid and mint oil. Initially wort was soured by citric acid to limit possibilities of sporeforming bacteria development.

This wort indicated about 11% of extract and total acidity of 0.14 g/100 cm^3. Wort was heated to 90°C, cooled to 26°C; it was inoculated with compressed baker's yeast in amount of 0.05 g/dm^3 then poured into bottles (4/5 of capacity) and closed with crown closure. After 24 hours of fermentation bottles were pasteurized for inactivation of yeast and then strongly cooled. Then they were opened, inoculated with identical quantity (5 cm^3) of liquid culture of one of the strains and again they were closed. The bottles were stored at 22°C and 6°C for period of 2 months. During that time, in 7-days intervals, bottles were opened and samples were taken out for chemical and microbiological determinations.

The following determinations were carried out:

– a number of lactic acid bacteria (*L. plantarum*) – applying a plate method, MRS medium of pH = 6.5; incubation of plates was carried out in 48 hours at the temperature of 28°C (optimum temperature for *L. plantarum* is 28-32°C),

– pH – in the glass electrode an calomel electrode system [A.O.A.C. 1995],

– total acidity – by potentiometric titration of a sample to pH 8.0 after previous degassing. The results were given in grams calculated as lactic acid in 100 cm^3 of the beverage,

– volatile acidity – after distillation with water vapor. The results were given in g/100 cm^3 as acetic acid [Drzazga 1996],

– reducing saccharides after inversion (total) – Luff–Schoorl method [Krełowska-Kułas 1993] after previous hydrolysis according to Clarget-Herzfeld. The results were given in g/100 cm^3 of a drink as inverted sugar,

– real extract – areometrically in residue after distillation of alcohol. The results were given in ° Blg [Drzazga 1996],

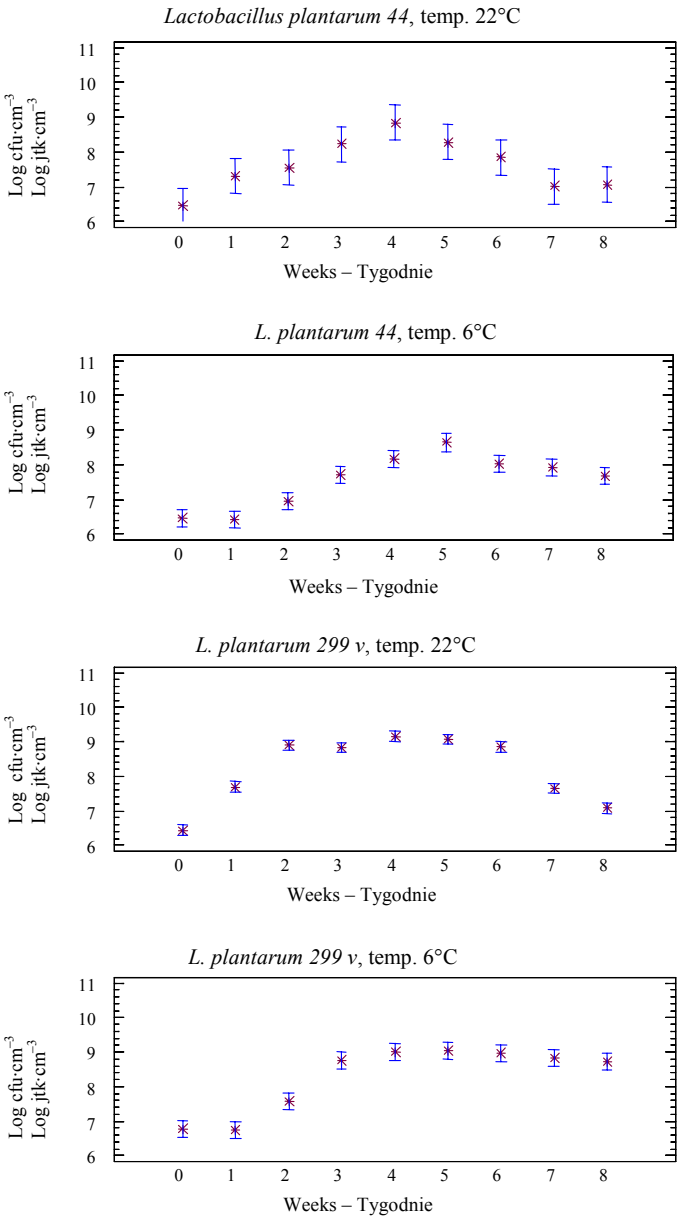
– alcohol content – aerometric method after distillation from a sample. The results were given in % v/v [A.O.A.C. 1995],

– the sensory analysis of the product was also carried out by board, while applying five – scores scale of nine quality levels. Overall quality was calculated as weighed average factor coefficient (coefficient ponderability): colour – 1, smell – 2, taste – 3.

Three series of experiments, each in two repetitions, were conducted. The results were elaborated statistically by means of Statgraphics Plus Ver. 4.1 program with application of One-way ANOVA – or Multifactor ANOVA ($\alpha = 0.05$) and NIR calculation according to Tuckey (as HSD-Honestly Significant Differences).

RESULTS AND DISCUSSION

The average number of lactic acid bacteria in the "malt beverage wort" directly after inoculation of bacteria amounted to about 3.16×10^6 cfu/cm^3. During bimonthly period of keeping "malt beverage" two characteristic periods were observed (Fig. 1). In the first



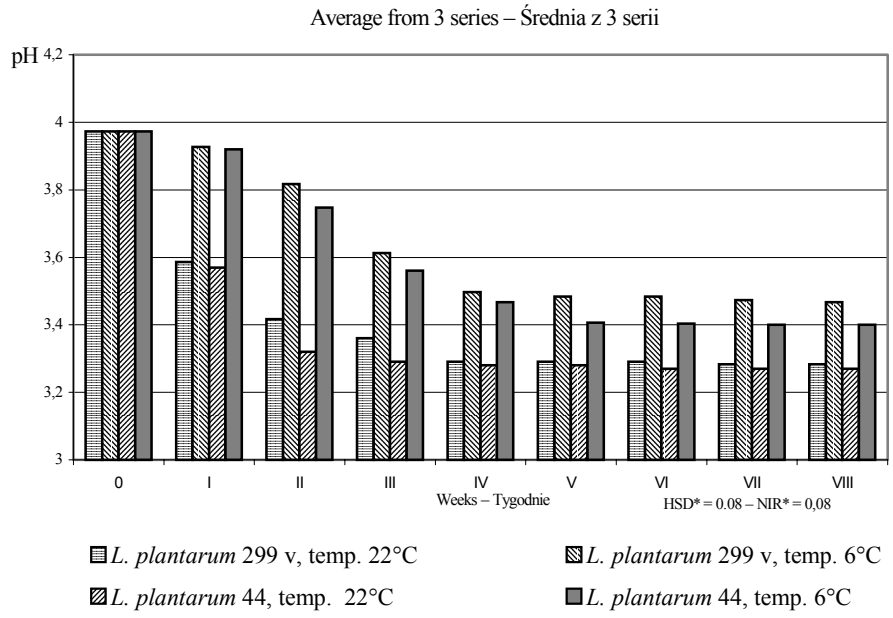
Average from three series.
Tukeys HSD Intervals, One-Way ANOVA, α = 0.05.
Średnia z trzech serii.
NIR wg Tuckey'a, jednoczynnikowa analiza wariancji, α = 0,05.

Fig. 1. Influence of the samples' storage time in the temperatures 6°C and 22°C on the number of cells (average from three series)
Rys. 1. Wpływ czasu przechowywania próbek w temperaturze 6°C i 22°C na liczbę komórek (średnia z 3 serii)

period of keeping beverages (2 weeks) in the temperature of 22°C, a bigger growth in the number of bacteria cells was observed in relation to samples kept in the temperature of 6°C. The bacterial strains stored in the temperature of 22°C reproduced intensively till the end of the fourth experiment week reaching the average 6.92×10^8 cfu/cm^3 for *L. plantarum 44* and 1.41×10^9 cfu/cm^3 for *L. plantarum 299 v*. In samples kept in the temperature of 6°C the biggest number of bacteria was identified after the fifth week of the experiment and amounted to the average of 4.37×10^8 cfu/cm^3 for *L. plantarum 44* and 1.07×10^9 cfu/cm^3 for *L. plantarum 299 v*.

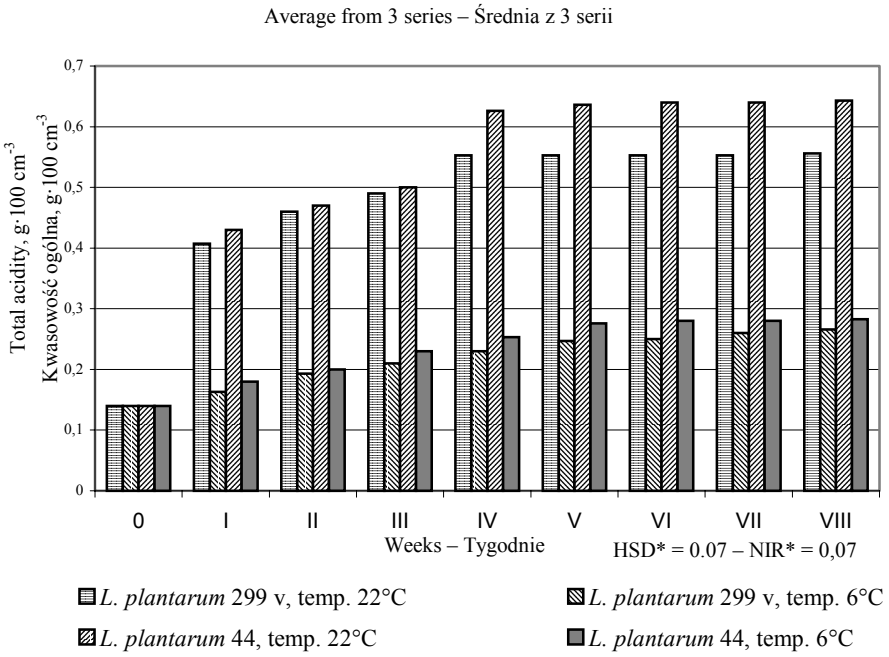
After this period the growth inhibition and gradual drop in number of cells was observed in the temperature of 22°C starting from the sixth week of keeping the samples, and from the seventh week in the temperature of 6°C.

Inhibition of bacteria life activity and a gradual drop in their number could result from the drop in pH value from 3.97 to 3.48 and 3.28 depending on temperature and a strain (Fig. 2) as well as on the increase of the total acidity (Fig. 3). It should be also taken into consideration that deficiency of some environmental constituents may influence growth and life activity, however in our conditions, the main factor was probably the decreasing pH of the environment.



*Tukeys HSD Intervals, Multifactor ANOVA, α = 0.05.
*NIR wg Tuckey'a, wieloczynnikowa analiza wariancji, α = 0,05.

Fig. 2. Influence of time, storage temperatures and the bacterial strain on pH value of the "malt beverage" (average from three series)
Rys. 2. Wpływ czasu, temperatury przechowywania i szczepu na pH „napoju słodowego" (średnia z 3 serii)



*Tukeys HSD Intervals, Multifactor ANOVA, α = 0.05.
*NIR wg Tuckey'a, wieloczynnikowa analiza wariancji, α = 0,05.

Fig. 3. Influence of time, storage temperature and the bacterial strain on total acidity of the "malt beverage" (average from three series)
Rys. 3. Wpływ czasu, temperatury przechowywania i szczepu na kwasowość ogólną „napoju słodowego" (średnia z 3 serii)

The important technological characteristic of the product of priobiotic properties is a minimal number of bacteria cells, which a particular product should contain. It should range from 10^5 to 10^6 in cm^3 of the product for viable cells of lactic acid bacteria. Such a quantity of bacteria allows to influence favorably the consumer's organism [Lee and Salminem 1995, Libudzisz 1999].

After the storage period was over the differences in the number of particular bacterial strains were discovered depending on the storage temperature (Fig. 1). In the samples of "malt beverage" stored in the temperature of 6°C a higher number of bacteria was observed than in the temperature of 22°C. In the temperature of 6°C in case of *L. plantarum 44* the average was 4.90×10^7 cfu/cm^3 and in case of *L. plantarum 299 v* – the average was 5.37×10^8 cfu/cm^3. In the samples stored in the temperature of 22°C this number amounted respectively to 1.15×10^7 cfu/cm^3 and 1.20×10^7 cfu/cm^3. It allows us to state that after two months of storage both bacterial strains are characterized by similar number of surviving cells dependent on the temperature applied.

Slightly smaller number of bacteria cells in samples stored in the temperature of 22°C after the storage is finished in relation to the samples stored in the temperature of

6°C may result from faster pH drop and the growth of total acidity in the temperature of 22°C what presumably may influence their survival ability.

The pH value of "malt beverage wort" directly after bacterial inoculation amounted to 3.98 (Fig. 2). After 3 weeks of storage in the temperature of 22°C it dropped to the average value of 3.28 and it maintained the same level further on. During storage in the temperature of 6°C, after 4 weeks of storing the pH – dropped to the value of approx. 3.5 and during further period of storing the changes were slight. The total acidity of the drink changed accordingly (Fig. 3).

The relatively low pH value (in relation to the observed total acidity of the product) was probably caused by low buffering of the beverage.

Considerable increase of titratable acidity was observed during the first four weeks of storing (Fig. 3). The highest increase in relation to the initial value of the drink (amounting to 0.14 g/100 cm^3) was observed in the first week of storage. In case of 6°C temperature the observed increase was in the range of 0.16-0.18 g/100 cm^3 depending on a bacterial strain but in the temperature of 22°C the number of total acidity amounted to 0.41-0.43 g/100 cm^3 and were growing to the fourth week of storing respectively within the range of 0.23-0.25 g/100 cm^3 and 0.55-0.62 g/100 cm^3. It may result from a wide availability of nutrients and lack of inhibitory influence, among other things, of substrates produced during that process. After 5th week of storage the differences observed were contained within the range of a mistake. Probably it could be caused by the inhibition of bacterial cells metabolic activity.

These observations confirm the results of Duran-Quintan et al. [1999], who examined the influence of different temperatures on lactic acid fermentation of green olives. The authors stated that the ability of environmental souring depends on the temperature of the process. Bacteria from the *L. plantarum species* acting in the temperature of 15°C demonstrated stronger growth and bigger acid – forming ability in relation to the ones stored in lower temperatures (9°C and 12°C).

Table 1. Chosen ingredients of the "malt beverage" before bacterial inoculation and after the storage period ends (average from three series)
Tabela 1. Wybrane składniki "napoju słodowego" przed zaszczepieniem bakteriami oraz po zakończeniu okresu przechowywania (średnia z 3 serii)

Ingredient Składnik	Before inoculation Przed zaszczepieniem	After storage Po zakończeniu przechowywania			
		L. plantarum 44		*L. plantarum 299 v*	
		temp. 22°C	temp. 6°C	temp. 22°C	temp. 6°C
Real extract, °Blg Ekstrakt rzeczywisty, °Blg	9.8	8.4	9.0	8.6	9.2
Alcohol, %v/v Alkohol, % obj	0.40	0.47	0.47	0.47	0.40
Volatile acidity, g/100 cm^3 Kwasowość lotna, g/100 cm^3	trace ilości śladowe	0.040	0.023	0.032	0.020
Total sugars, g/100 cm^3 Cukry ogółem, g/100 cm^3	7.72	6.43	7.13	6.60	7.17

Table. 2. Values of sensory analysis of "malt beverage" during storage, 5- points score (average from 3 series)
Tabela. 2. Wyniki liczbowe analizy sensorycznej „napoju słodowego" podczas przechowywania, skala pięciopunktowa (średnia z 3 serii)

Strain Szczep	***Lactobacillus plantarum 299 v***															
Week Tydzień	I		II		III		IV		V		VI		VII		VIII	
Temperature Temperatura	22°C	6°C	22°C	6°C	22°C	6°C	22°C	6°C	22°C	6°C	22°C	6°C	22°C	6°C	22°C	6°C
Colour Barwa	2.8	3.2	3.2	3.7	3.0	3.3	3.5	3.8	3.7	3.8	3.7	3.7	3.8	3.8	3.7	4.0
Smell Zapach	3.0	3.5	3.8	3.5	3.7	4.2	3.8	4.2	3.5	4.3	3.8	4.2	3.7	4.2	3.5	4.0
Taste Smak	3.8	3.5	3.7	3.8	3.5	3.8	3.3	3.8	3.5	3.7	3.5	4.0	3.7	4.0	3.7	3.7
Overall quality Ocena ogólna	**3.5**	**3.5**	**3.6**	**3.7**	**3.5**	**3.9**	**3.5**	**3.9**	**3.5**	**3.8**	**3.6**	**4.0**	**3.7**	**4.0**	**3.6**	**3.8**
Strain Szczep	***Lactobacillus plantarum 44***															
Week Tydzień	I		II		III		IV		V		VI		VII		VIII	
Temperature Temperatura	22°C	6°C	22°C	6°C	22°C	6°C	22°C	6°C	22°C	6°C	22°C	6°C	22°C	6°C	22°C	6°C
Colour Barwa	3.5	3.8	3.5	4.0	3.7	4.0	4.0	4.5	4.0	4.0	3.8	4.2	4.0	4.2	3.8	4.2
Smell Zapach	4.0	3.7	3.5	4.0	3.8	4.2	4.0	4.2	4.0	4.0	4.0	4.0	4.0	4.0	4.0	4.0
Taste Smak	3.7	3.8	3.8	3.8	4.0	4.0	3.7	4.0	3.5	3.9	3.5	3.5	3.3	3.3	3.3	3.3
Overall quality Ocena ogólna	**3.7**	**3.8**	**3.7**	**3.9**	**3.9**	**4.0**	**3.8**	**4.1**	**3.7**	**3.9**	**3.7**	**3.7**	**3.6**	**3.6**	**3.5**	**3.6**

Different quantities of lactic acid produced by the examined lactic bacterial strain result from their varied dynamics of sugar fermentation. Moneta and Libudzisz [2000] stated that the quantity of the produced lactic acid is a bacterial strain characteristics but not a species one. *L. acidophilus Ch – 5* produced 0.6% of lactic acid during a 24 – hour fermentation while *L. acidophilus A92* strain as much as 2.3%.

Additionally the presence of acetic acid, apart from lowering pH environment may cause an intracellular protein denaturation and pH value reduction inside the cells of lactic acid bacteria [Klewicka and Libudzisz 1998]. However in our case the content of volatile acids was slight and amounted to 0.02-0.04 g/100 cm^3.

It was stated that the production and storing conditions of the "malt beverage" as well as the bacterial strain used caused slight differences in the final sugar content in the product (Table 1). After the storage period the decrease in total sugar content was observed in relation to the beverage samples before inoculation. The average total sugar content in the sample before bacterial inoculation amounted to 7.72 g/100 cm^3. In samples after storing in the temperature of 22°C it amounted to 6.60 g/100 cm^3 in case of *L. plantarum 299 v* and 6.43 g/100 cm^3 in case of *L. plantarum 44.* The total quantity of total sugar in samples stored in the temperature of 6°C was bigger in comparison to the ones stored in temperature of 22°C and amounted to approximately 7.15 g/100 cm^3, what results from the quantity of the lactic acid obtained – that is total acidity.

Beverages obtained were characterized by a good sensory quality but the beverage obtained with participation of the *L. plantarum 44* appeared to be too acid after seven weeks of storage and it might be desirable to increase their sweetness or to use lactic acid bacteria with lower ability of lactic acid production (Table 2).

CONCLUSIONS

It is possible to obtain a drink, of vegetable origin ("malt beverage") containing viable lactic acid bacteria, majority of which maintain viability at least for two months of storage (in temperatures 22°C and 6°C). The number of bacteria after completion the period of storage ranged from 10^7 to 10^8 cfu/cm^3 depending on the temperature and strain that is on the level of at least equal to the one required for probiotic products.

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PRÓBY OTRZYMANIA NAPOJU ZAWIERAJACEGO ŻYWE BAKTERIE FERMENTACJI MLEKOWEJ ORAZ OKREŚLENIE ICH PRZEŻYWALNOŚCI W WYBRANYCH TEMPERATURACH

Streszczenie. Celem pracy było uzyskanie „napoju słodowego" zawierającego żywe kultury bakterii fermentacji mlekowej z gatunku *Lactobacillus plantarum* oraz określenie przeżywalności tych bakterii w trakcie 2-miesięcznego przechowywania w temperaturze 22°C i 6°C. Po zakończeniu okresu przechowywania stwierdzono liczbę komórek bakterii w przedziale 10^7-10^8 jtk/cm^3, czyli na poziomie wyższym niż minimalna ilość bakterii mlekowych wymaganych dla produktów probiotycznych. Zaobserwowano m. in. obniżenie wartości pH z 3,97 do 3,46 i 3,28 w zależności od temperatury przechowywania oraz wzrost kwasowości ogólnej z 0,14 do 0,27 i 0,60 g/100 cm^3 produktu. Po okresie przechowywania (i w trakcie) stwierdzono dobrą jakość sensoryczną kwasu słodowego.

Słowa kluczowe: bakterie fermentacji mlekowej, probiotyki, zdolność przeżywania, przechowywanie, kwas chlebowy, napój słodowy

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