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ASSESSMENT OF THE LACTICASEIBACILLUS RHAMNOSUS LOCK 900 STRAIN FOR THE PRODUCTION OF PROBIOTIC BEETROOT JUICE®

Ocena przydatności szczepu Lacticaseibacillus rhamnosus LOCK 900 do produkcji probiotycznego soku z buraka ćwikłowego®

Lactic acid bacteria play an important role in the development of new functional products. The addition of probiotic bacteria strain can give the product features of functional food. The aim of the study was to assess the possibility of using the probiotic Lacticaseibacillus rhamnosus LOCK 900 strain for the production of functional red beet juice (Beta vulgaris) with a satisfactory sensory quality and determination of the health safety of the proposed product in terms of the content of biogenic amines. In our research it was found that the number of Lb. rhamnosus LOCK 900 bacteria strain until the 12th day of storage at 4°C was at a high level, above 8 log cfu/ml and was suitable for probiotic products, in accordance with FAO/WHO recommendations. The overall quality of fermented beet juice supplemented with LOCK 900 strain stored at 4 °C was high until the end of storage period. It is possible to use the Lacticaseibacillus rhamnosus LOCK 900 strain to obtain fermented beetroot juice with the number of cells in accordance with the FAO/WHO recommendations for probiotic products.

Key words: probiotic, beet juice, *Lactobacillus*, starter culture, functional food.

Bakterie kwasu mlekowego odgrywają ważną rolę w rozwoju nowych produktów funkcjonalnych. Dodanie szczepu bakterii probiotycznych pozwala na zaliczenie produktu do żywności funkcjonalnej. Celem badania była ocena możliwości wykorzystania szczepu probiotycznego Lacticaseibacillus rhamnosus LOCK 900 do produkcji fermentowanego soku z buraka ćwikłowego (Beta vulgaris), charakteryzującego się dobrą jakością sensoryczną i bezpieczeństwem zdrowotnym pod względem zawartości amin biogennych. Stwierdzono, że liczba bakterii szczepu *Lb. rhamnosus* LOCK 900 do 12 dnia przechowywania w 4°C była powyżej 8 log jtk/ml i była zgodna z wymaganiami FAO/WHO dla produktów probiotycznych. Jakość ogólna fermentowanego soku z buraków z dodatkiem szczepu LOCK 900 przechowywanego w temperaturze 4 °C była wysoka do końca okresu przechowywania.. Istnieje możliwość zastosowania szczepu *Lacticaseibacillus rhamnosus* LOCK 900 do otrzymania fermentowanego soku z buraka ćwikłowego o liczbie komórek zgodnej z zaleceniami FAO/WHO dla produktów probiotycznych.

Słowa kluczowe: probiotyk, sok z buraka, *Lactobacillus*, kultura startowa, żywność funkcjonalna.

INTRODUCTION

Currently, people are looking for the products that promote well-being and health. Fermented milk drinks with the addition of bacteria are now popular probiotic foods. Some consumers, however, cannot consume them for a reason of allergies, lactose intolerance or cholesterol content. The fermented vegetable products (i.e. juices) made of vegetables, fruits, cereals and legumes can complement the offer [12, 15, 16]. The addition of probiotic bacteria strain can give the product features of functional food. The use of a starter culture of a selected bacterial strain ensures the proper course of fermentation. Starter cultures of lactic acid bacteria used for the production of such juices should be of good viablity in an acidic environment to the end of its use-by date consumption [2]. Lactic acid bacteria (LAB) play an important role in the development of new functional products. In addition to preserving food, LAB change the sensory characteristics of the product and inhibit the growth of pathogenic bacteria. Probiotic bacteria when added to food, give a beneficial effect on the host's organism by improving its intestinal ecosystem [24]. Currently, traditional food preservation processes such as fermentation are becoming more popular. An attractive healthy product may be a fermented beet juice. Beetroot is seen as a food with a positive effect on health, as it contains a large number of antioxidants. The consumption of beetroot may contribute to lowering the risk of cardiovascular diseases and cancer [13]. The addition of probiotic bacteria strains to beet juices may increase the attractiveness of the products offered to consumers. Obtaining the viability of strains of

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probiotic microorganisms at an appropriate level in products derived from red beet makes it possible to classify them as functional food.

The aim of the study was to assess the possibility of using the probiotic *Lacticaseibacillus rhamnosus* LOCK 900 strain for the production of functional red beet juice with a satisfactory sensory quality and determination of the health safety of the proposed product in terms of the content of biogenic amines.

MATERIAL AND METHODS

The research material was juice made of roots of red beet (*Beta vulgaris L. ssp. vulgaris*, Wodan) from the crops of the Warsaw University of Life Sciences, without the use of plant protection products. Beetroots were washed, sliced, then juice was pressed using Kenwood JE 500, Germany juice extractor. Saccharose was added in the amount of 2% of the juice volume, then the juice was pasteurised (90°C, 15 minutes), chilled to 40°C and juice was inoculated with probiotic starter culture *Lb. rhamnosus* LOCK 900 and kept in the cooling temperature.

The pure probiotic strain LOCK 900 (strain deposit number: CP005454) was obtained from a collection from the Pure Culture Collection of the Technical University of Łódź in Poland [1]. Preparation of the probiotic strain consisted of activating frozen bacteria, suspending them in food broth and then inoculating the juice with them [19]. The inoculum before being added to the juice was approximately 9.0 log cfu/mL.

The number of *Lb. rhamnosus* LOCK 900 bacteria was determined adopting a plate method on the MRS Agar (BIOCAR Diagnostic) at 37°C for 48 h.

The pH value was measured using digital pH meter (Lab 860 Schott Instruments) equipped with a pH electrode.

Determining of biogenic amines with using High-Performance Liquid Chromatography (Alliance Separation Module 2695, Waters Alliance, Milford, USA) in reversed phase (RP-HPLC). Chromatographic separation was perform using column: Kromasil C18 (5 µm, 250 mm × 4,6 mm ID, with proper precolumn precolumn). Column was maintained at 25ºC. Analytes were detected at the wavelength of 254 nm. The sampler temperature was 4ºC, the mobile phase (acetonitryl : water) flow rate was 1 cm^3 /min. The gradient elution program was as follows: [%]: 1 min – 35:65, 10 min – 20:80, 12 min – 10:90, 16 min – 0:100, 23 min – 0:100. The retention time (RT) for the determination of one sample of biogenic amines was 23 min. The individual amine content results were calculated with the equation: y = cAB (Tyr, Pu, Hi) /cI × b and are given in mg/kg.

For sensory evaluation QDA method was applied. An unstructured, linear graphical scale (0–10 conventional units) was used (ISO 13299:2016-05 ISO 8586:2014-03). In trained person panel discussion 13 descriptors and overall quality were chosen [9,10].

The data were evaluated statistically using STATISTICA ver. 12 (Statsoft, Isc. 2011) and Microsoft Excel 2007 program. The results were analysed using one-way analysis of variance and Tukey test. The significance of differences between means were calculated at the level P= 0.05.

RESALTS AND DISCUSSION

In the first stage of the research to ensure growth of *Lacticaseibacillus rhamnosus* LOCK 900 strain in environment of beet juice the addition of sugar (sucrose) was checked. As a result of juice fermentation (37 °C for 24 hours) with the *Lb. rhamnosus* LOCK 900 strain, the number of bacteria increased more than 1 logarithmic level to the values for juice without and with sucrose respectively: 8.82 log cfu/ml and 8.62 log cfu/ml.

In our research, sensory evaluation was used as a criterion for product selection by the consumer. The general quality of the juice with the addition of sucrose was statistically significantly higher than the juice without added sugar, which could be influenced by higher scores of positive discriminants: sweet flavour and sweet odour for this variant (Fig. 1). The variant of the juice with added sugar was selected for further research.

The next stage of the research was to evaluate survival of the *Lb. rhamnosus* LOCK 900 in fermented beet juice. The beetroot juice was fermented at 37 °C and stored for 16 days at 4 °C and 8 °C. The storage time and temperature influenced the viability of the tested strain and the pH. During the storage of the juice at 4 °C, there was a significant increase in the number of bacteria of the tested strain up to the 4th day of storage, after which the number of bacteria decreased (Fig. 2a). A probiotic product should contain a sufficient number of living, defined, health-promoting microorganisms, the general recommendation is 10^6 cfu/g. [7]. In our research it was found that the number of LOCK 900 bacteria until the 12th day of

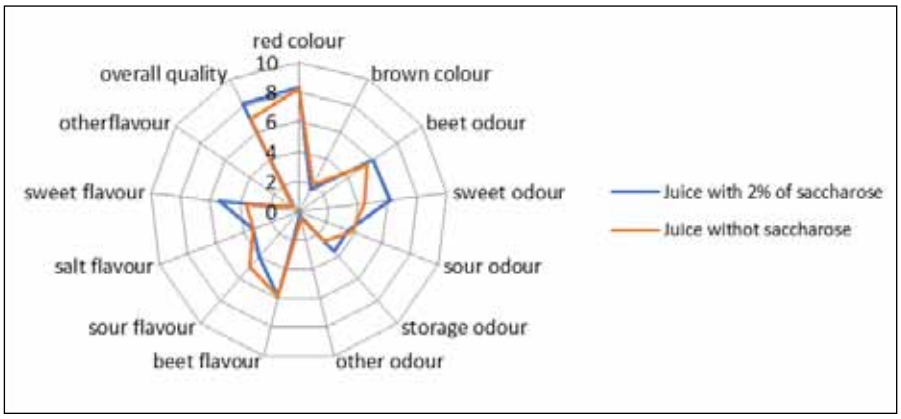


Fig. 1. Evaluation of intensity of sensory attributes of fermented beet juice without saccharose and with 2% saccharose added during storage (QDA method, n=16).

Rys. 1. Ocena intensywności wyróżników sensorycznych fermentowanego soku z buraka ćwikłowego bez oraz z dodatkiem 2% sacharozy (metoda QDA, n=16).

Source: The own study

Źródło: Badania własne

storage at 4°C was at a high level, above 8 log cfu/ml (Fig. 2A) and was suitable for probiotic products, in accordance with FAO/WHO recommendations. During storage at 4°C a significant decrease in pH on day 4 was observed, which proves the presence of live cells of lactic acid bacteria, which by consuming the sugars contained in the food matrix (beet juice) cause acidification of the environment (Fig. 2B). The juice stored at 8 °C also showed an increase in the number of bacteria up to the 4th day of storage, although not statistically significant. Until the 8th day of storage, the number of probiotic bacteria was above 8 log cfu/ml (Fig. 2A), while after 12 days no viable *Lb.rhamnosus* LOCK 900 cells were found. At a higher temperature (8°C), sugar is metabolized faster by LOCK 900 bacteria, hence their reduced survival is observed. Baráth et al (2004) also found a reduction in the number of *Lb. curvatus* 2770 and *Lb. casei pseudo plantarum* from the values of 8.85 and 8.0 log cfu/ml, respectively, to a level of about 6.0 log cfu/ml during the refrigerated storage of beet juice for 28 days [3]. Diversified survival of different LAB strains was also demonstrated in research of [25]. They found good survival of the *Lb. plantarum* and *Lb. acidophilus*, but did not find the presence of viable *Lb. plantarum* cells after 2 weeks of storage of red beet juice at 5°C. It can be assumed that the viability of bacteria in the fermented juice during storage may be dependent on the strain used. The viability of bacterial strains may also be related to the food matrix. Yuasa et.al. (2021) used *L. plantarum* SI-1 and *L. pentosus* MU-1 in citrus juice fermentation. The viable cell counts tended to be lower in fermented citrus *Haruca* juice than in mandarin and orange juices for both bacteria [26].

The overall quality of the beet juice supplemented with LOCK 900 strain stored at 4°C was high until the end of storage (Fig. 3). The highest general quality of juice (7.33 c.u.) was observed after 8 days, which could have been influenced by an increase, although not statistically significant, of positive qualitative characteristics: beetroot taste, sweet taste, sweet smell, red colour and clarity. The taste and smell of beetroot were best assessed after fermentation (6.63 c.u. and 6.23 c.u. respectively). The juice stored at 8°C was of satisfactory sensory quality until the 8th day of storage (Fig. 3). There were no statistically significant differences between the examined discriminants during storage. The overall quality of the juice decreased on the 16th day of storage to 4.9 c.u. and the product was not sensory acceptable.

The basic requirement for food is its safety. According to the definition of Codex Alimentarius food safety is defined as "assurance that food will not cause harm to the consumer when it is prepared and / or eaten according to its intended use" [6]. Biogenic amines occurring in food may pose a threat to human health therefore monitoring their presence is very important. The presence of amines was found in fresh and preserved vegetables and fermented plant juices [11, 14, 17, 26] and their type may depend on the strain of bacteria [21]. There are many factors influencing the formation of biogenic amines, including temperature, pH, substrate availability, salt and sugar content. In products containing biogenic amines, the microorganisms responsible for their formation have also been identified [8].

Histamine is considered to be the most toxic amine found in food. No histamine was found in the juice after

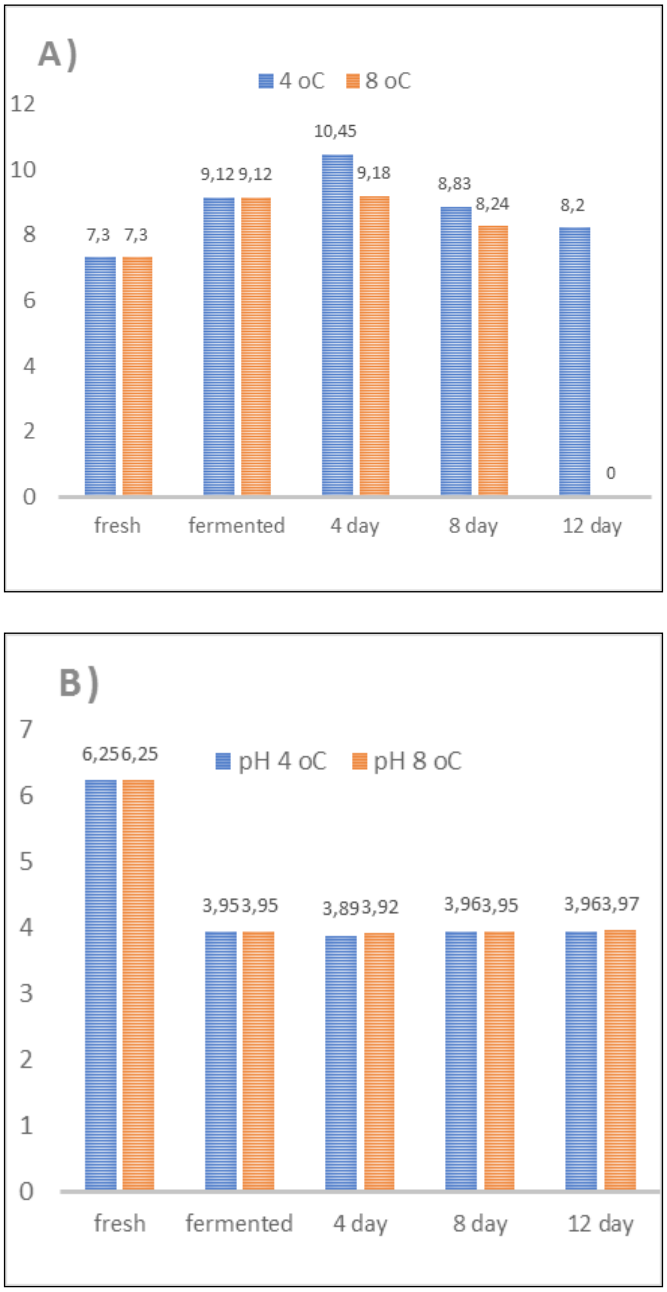


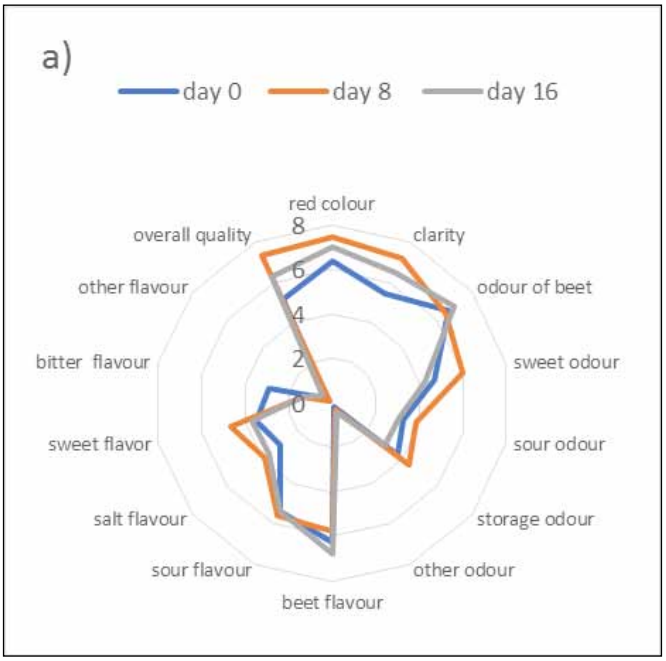
Fig. 2. Mean counts of L. rhamnosus LOCK 900 strains [log cfu/ml] (A) and pH (B) in fermented beet juice with saccharose (2%) added during storage in temperature 4°C and 8°C.

Rys. 2. Średnie wartości liczby bakterii L. rhamnosus LOCK 900 [log jtk/ml] (A) i pH (B) w fermentowanym soku z buraka ćwikłowego z dodatkiem sacharozy (2%) przechowywanego w temperaturze 4°C i 8 °C.

Source: The own study

Źródło: Badania własne

fermentation and during storage in fermented beet juice (Tab. 1). Fruits and fruit juices are particularly rich in putrescine [4,16, 22]. According to some authors, cooking processes and heat treatments can influence polyamine contents [5, 20, 23]. The regulation of European Union Commission 1019/2013 established limits for histamine (20-40 mg/100g) in fishery



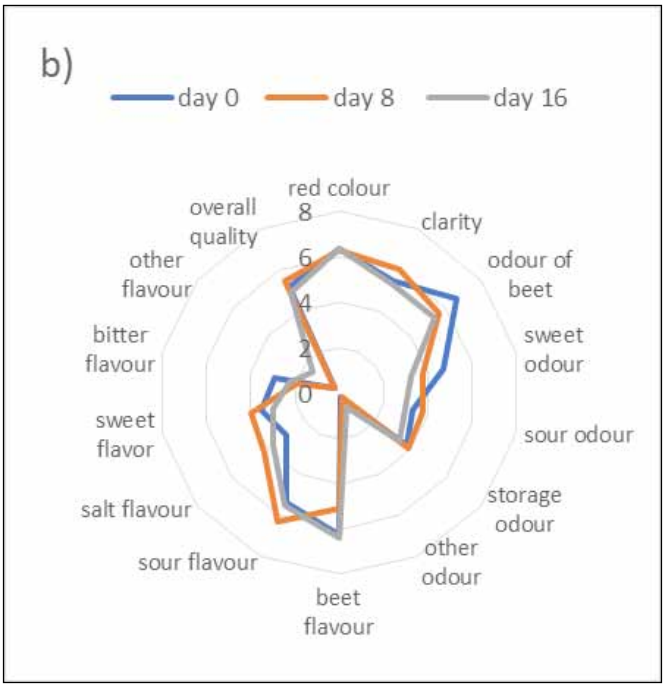


Fig. 3. Evaluation of intensity of sensory attributes of beet juice with 2% saccharose addition after fermentation and during 16 days storage: a) at 4 oC, b) at 8 oC (QDA method, n=16).

Rys. 3. Ocena intensywności wyróżników sensorycznych soku z buraka ćwikłowego z 2% dodatkiem sacharozy po fermentacji oraz podczas 16 dni przechowywania : a) 4 oC, b) 8 oC (metoda QDA, n=16).

Source: The oun study

Źródło: Badania własne

products. The content of putrescine and tyramine in the tested beetroot juice was at a low level after pasteurization and during the entire storage period.

Table 1. Biogenic amine contents in fermented beet juice with saccharose (2%) added [mg/kg]

Tabela 1. Zawartość amin biogennych w fermentowanym soku z buraka ćwikłowego z dodatkiem 2% sacharozy [mg/kg]

Juice/sok		Histamine	Tyramine	Putrescyne
Fresh		n.d.	n.d.	3,17±0,61
Pasteurised		n.d.	1,86±0,23	1,14±0,22
Fermented		n.d.	1,86±0,23	1,18±0,23
Temp.	Days			
4°C	8	n.d.	2,00±0,24	1,63±0,31
	16	n.d.	2,22±0,27	1,45±0,28
8°C	8	n.d.	3,42±0,42	3,27±0,63
	16	n.d.	1,88±0,23	1,88±0,23

LOD=0,5 mg/kg, LOQ=1,0 mg/kg, n.d. – not detected (< LOD)

Source: The oun study

Źródło: Badania własne

CONCLUSIONS

It was found that the storage time and temperature influenced the microbiological quality of the fermented beetroot juice inoculated with *Lacticaseibacillus rhamnosus* LOCK 900 with the addition of 2% sucrose. Better survival of *Lacticaseibacillus* rhamnosus LOCK 900 cells was found during the two-week storage of fermented beetroot juice at 4 than 8 °C. The content of biogenic amines (histamine, tyramine, putrescyne) was low during the production and storage of the fermented beetroot juice. Taking into account the sensory quality, the juice storage time at 4˚C and 8˚C is no longer than 8 days.

It is possible to use the *Lacticaseibacillus rhamnosus* LOCK 900 strain to obtain fermented beetroot juice with the number of bacterial cells in accordance with the FAO/WHO recommendations for probiotic products. Further research is necessary to optimize the parameters of the juice production process and the selection of starter cultures.

WNIOSKI

Stwierdzono, że czas i temperatura przechowywania mają wpływ na jakość mikrobiologiczną fermentowanego soku z buraka ćwikłowego zaszczepionego *Lacticaseibacillus rhamnosus* LOCK 900 z dodatkiem 2% sacharozy. Podczas dwutygodniowego przechowywania fermentowanego soku z buraka ćwikłowego stwierdzono lepszą przeżywalność komórek *Lacticaseibacillus rhamnosus* LOCK 900 w temperaturze 4 niż 8°C. Zawartość amin biogennych (histaminy, tyraminy, putrescyny) była na niskim poziomie podczas produkcji i przechowywania fermentowanego soku z buraka ćwikłowego. Biorąc pod uwagę jakość sensoryczną, czas przechowywania soku w temperaturze 4˚C i 8˚C wynosi nie dłużej niż 8 dni.

Istnieje możliwość zastosowania szczepu *Lacticaseibacillus rhamnosus* LOCK 900 do otrzymania fermentowanego soku z buraka ćwikłowego o liczbie komórek bakterii zgodnej z zaleceniami FAO/WHO dla produktów probiotycznych. Niezbędne są dalsze badania nad optymalizacją parametrów procesu produkcji soku i doborem kultur startowych.

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