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APPLICATION OF *LACTOBACILLUS CASEI* O12 STRAIN FOR THE PRODUCTION OF PROBIOTIC TOMATO JUICE WITH ADDITION OF SEA BUCTHORN®

Zastosowanie szczepu *Lactobacillus casei* O12 do produkcji probiotycznego soku pomidorowego z dodatkiem soku z owoców rokitnika®

Key words: *Lactobacillus*, tomato, sea buckthorn, fermentation, probiotics.

The aim of the work presented in the article was to evaluate the application of Lactobacillus casei O12 strain for the production of probiotic tomato juice with 3% addition of sea buckthorn. Fermentation was carried out with potentially probiotic Lactobacillus casei O12 strain isolated from fermented cucumbers. Fermentation carried out at 37ºC for 18 hours. The viable cell count of Lb. casei O12 at the end of the storage at 4°C and 15°C was 9.3 and 9.4 log CFU/mL respectively, and was similar to probiotic foods. It was found that fermented tomato juice with 3% addition of sea buckthorn juice stored at 4°C for 16 days and 8 days at 15°C have a satisfying sensory quality.

Słowa kluczowe: *Lactobacillus*, pomidor, owoc rokitnika, fermentacja, probiotyki.

Celem pracy przedstawionej w artykule była ocena zastosowania szczepu Lactobacillus casei O12 do produkcji probiotycznego soku pomidorowego z dodatkiem 3% soku z owoców rokitnika. Fermentację przeprowadzono w temperaturze 37°C przez 18 godzin z zastosowaniem potencjalnie probiotycznego szczepu Lactobacillus casei O12 wyizolowanego z kiszonych ogórków. Liczba żywych komórek Lb. casei O12 pod koniec okresu przechowywania w 4°C i 15°C wynosiła odpowiednio: 9.3 and 9.4 log jtk/mL i była odpowiednia, jak dla żywności probiotycznej. Stwierdzono, że sok pomidorowy z dodatkiem 3% soku z owoców rokitnika przechowywany w temperaturze 4°C przez 16 dni i 8 dni w temperaturze 15°C ma zadowalającą jakość sensoryczną.

INTRODUCTION

Recently, there has been an increase in consumer interest in non-diary probiotic products. Vegetables and fruits are characterized by high nutritional and health-promoting value, with a low caloric value at the same time. Many efforts have been taken in the development of novel functional probiotic beverages. The composition of plant-based products indicates that they can be a potentially good carrier of probiotic bacteria. Recently, some fermented vegetable or fruit juices with lactic acid bacteria have been developed as probiotic foods [13, 21, 24, 28, 33, 34].

Tomato is a popular raw material that has a high nutritional value, with a low caloric value. Tomatoes and tomato products have health-promoting properties and contain beneficial nutrients and antioxidants, including Vitamins A and C, α-lipoic acid, lycopene, choline, folic acid, β-carotene, lutein and minerals. Health benefits of raw tomatoes and tomato juice have been reported in many studies [1, 3, 10].

Sea buckthorn (*Hippophae rhamnoides* L.) is a berry that, due to its high content of natural antioxidants (including flavonoids, tannins, phenolic acids, ascorbic acid, carotenoids and tocopherols) can be a valuable addition to food products [5, 11, 32]. According to Sireswar et al. (2020) the phenolic compounds of sea buckthorn may have additive and protective effects on *Lactobacilus rhamnosus* GG [29].

The sensory attributes of sea buckthorn can influence the final sensory quality of the of the food product [5]. Therefore the balance between the benefits to human health and the consumer sensory acceptance it should be considered.

The FAO and WHO recommends the use of probiotic bacteria derived from the human gastrointestinal tract [8]. Microorganisms with potentially probiotic properties can also be obtained from fermented and unfermented products of plant and animal origin. However, despite the evidence arising from the history of safe long-term use of traditional food products containing such microorganisms, further research are needed to confirm their health benefits and safety. Recently, in many works it was proved that strains of potentially probiotic bacteria obtained from traditional fermented foods have been successfully used in the production of functional foods [2, 4, 12, 18, 31]. The aim of the study was to evaluate the use of *Lactobacillus casei* O12 strain isolated from fermented cucumbers for the production of probiotic tomato juice with 3% addition of sea buckthorn.

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MATERIAL AND METHODS

The research material consisted of fresh, oblong, almost seedless tomatoes (*Solanum lycopersicum* L.) of the ground cultivar. Sea buckthorn (*Hippophae rhamnoides* L) juice, producer Polska Róża, distributor Bio Planet S.A. Wilkowa Wieś 7, 05-084 Leszno, Poland came from organic farming. The *Lactobacillus casei* O12 strain was isolated from fermented cucumbers and came from the collection of Departament of Food Gastronomy and Food Hygiene, Institute of Human Nutrition Sciences. The strain showed some traits of probiotic properties, which allowed it to be classified as a potential probiotic [35].

Preparation of the tomato juice with addition of sea buckthorn juice

Fresh tomatoes were stored in wooden baskets at room temperature in the dark for 24 hours. They were cleaned and thoroughly washed with tap water, and then the juice was squeezed out of them using a Kenwood juicer (England). 1% sucrose and the addition of pasteurized, sea buckthorn fruit juice in the amount of 3% were added to the tomato juice. The juice was pasteurized at 95°C for 5 minutes. The juice obtained in this way was poured into sterilized conical flasks closed with a cotton bud. After cooling to room temperature, the juice was inoculated with a previously centrifuged culture of potentially probiotic bacteria *Lactobacillus casei* O12. The inoculum concentration before being added to the juice was about 9.0 log CFU/mL. Fermentation of the juice lasted 18 hours and was carried out at a temperature of 37°C. After fermentation the juices were storage at 4°C and 15°C for 16 days. The fermented samples were collected at 0, 4, 8, 12, 16 days of storage for microbiological, pH and sensory analysis.

Microbiological analysis

The strains were stored in frozen culture at -80°C in media with 20% glycerol. The preparation of the strain consisted of activating frozen bacteria and passaging it on MRS broth (Biokar Diagnostic, France). After incubation at 37°C the culture medium was centrifuged and replaced with food broth. Then the juice was inoculated with bacteria.

Microbiological determinations performed according to ISO 15214:2002 standard [14]. The measurement were carried out with a traditional plate method using a MRS agar (Merck, Germany), like a dedicated culture media for a Lactic Acid Bacteria (LAB). Serial dilution of juices were prepared. Aliquots of 1 mL of dilution were plated in MRS agar plates then the plates were incubated under anaerobic conditions at 37 °C per 48 h. Plates containing 30–300 colonies were counted. The viable counts of potential probiotics were expressed as log CFU/mL.

The pH analysis

The pH values were measured using a pH meter (Elmetron CP 501, Poland) taking into account the temperature of measurement. The accuracy was 0.001. The pH values were performed in triplicate.

Sensory evaluation

The sensory quantitative descriptive analysis method was used with an unstructured, linear graphical scale: 100 mm was converted to numerical values (0–10 conventional units, where 0 means absence or very low intensity of the descriptor and 10 means very high intensity of the descriptor) [16]. Sensory analyses were performed by the 9 person trained according to the ISO 8586:2012 standard [15]. A list of 16 sensory attributes with definitions were used by the panel. The evaluation was repeated twice so as each mean result was based on minimum 18 unitary results. All the samples for evaluation were individually coded with three-digit codes and were delivered in at random sequence. The tests were carried out at a room temperature, in the place with the day light.

Statistical analysis

The statistical analysis of the results was performed using the Statistica 13.3 software (StatSoft, Kraków, Poland). The multivariate analysis of variance (ANOVA) was used. Fisher's NIR test was used to compare post-hoc means. The difference was considered statistically significant when $p < 0.05$.

RESULT AND DISCUSSION

The growth of probiotic bacteria during fermentation, and their viability during storage is important for the quality and stability of the product. Changes in the viable counts of potential probiotic strain after fermentation and during storage are presented in Fig. 1. The initial viable cell counts after fermentation was high and was 8.9 log CFU/mL and after storage it increased respectively for juice stored at 4°C and 15°C: 9.3 log cfu/mL and 9.4 log CFU/mL. During storage the number of *Lb. casei* O12 strain showed an increase in both fermented juices, especially in the early stage of fermentation (up to 4 days). At 15°C, greater bacterial growth was observed than at 4°C, which may be due to the greater metabolic activity of the bacteria. The higher temperature created more favorable conditions for the growth and development of bacteria, but the difference in the number of bacterial cells between juices stored at different temperatures was statistically insignificant ($p>0.05$). The survival of bacterial strains during storage may be related to the type of strain used. Yoon et al. (2004) also found high survival rates of the strains studied: *Lactobacillus acidophilus* LA39, *Lactobacillus casei* A4, *Lactobacillus plantarum* C3, *Lactobacillus delbrueckii* D7 during storage for 4 weeks at 4°C of fermented tomato juice [33]. The viable cell counts of *Lactobacillus acidophilus* LA39 and *Lactobacillus delbruecki* D7 didn't decrease during refrigerating storage and was $1.4x10^9$ and 8.1×10^8 respectively on the last day of storage. The viable cell counts *Lactobacillus casei* A4 and *Lactobacillus plantarum* C3 decreased slightly during storage, but was still at a high level above 10^6 [33]. In the research of King et al. (2007) tomato juice was fermented with *Lactobacillus acidophilus* BCRC 10695 at 37°C for 80 hours. After 10 weeks of storage in 4°C, the number of bacteria of this strain decreased from 10^9 CFU/mL after fermentation to approx. 10^4 log CFU/mL, which could be associated with the low pH of the juice [17]. The survival of lactic acid strains may also be influenced by the interaction of the strains used between themselves, culture condition, oxygen content, final acidity of the product, concentration of lactic acid and acetic acid [6, 30, 34].

Survival is also related to the type of matrix (raw material from which the juice was obtained) [20, 27]. Sheehan et al.(2007) found that the survival rate of *Lactobacillus paracasei* in pineapple juice was higher than in cranberry juice despite the identical pH of both juices (pH 3.5) [26]. The study by Min et al. (2019) does a review on probiotic food products of non-dairy origin: cereals, fruits, vegetables, soy and meat products and states that the food matrix play an important role in the growth of microorganism and confirms that survival of cells in such matrices are strain dependent [20]. The steps occurring in the technological process with fruits and vegetables, such as peeling and cutting release cellular content and create ideal conditions for microbial growth [25]. Based on the obtained own results, it can be concluded that tomato juice with 3% addition of sea buckthorn juice is a good environment for the growth of *Lactobacillus casei* O12 strain.

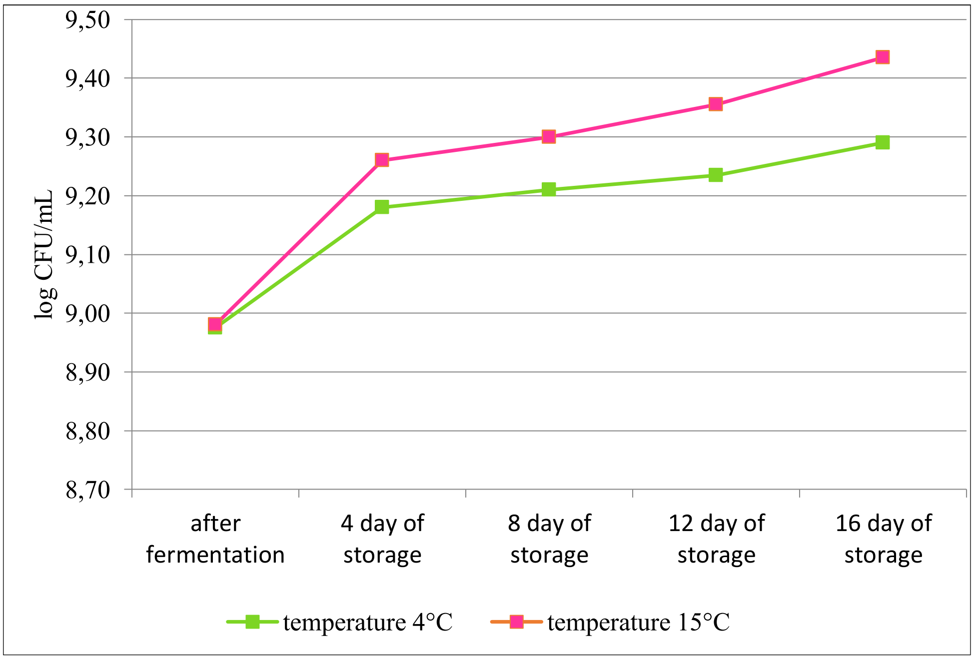

Fig. 1. Changes in Lb. casei O12 counts of fermented tomato juice with 3% addition of sea buckthorn during storage.

Rys. 1. Zmiany liczby komórek Lb. casei O12 w fermentowanym soku pomidorowym z 3% dodatkiem soku z owoców rokitnika podczas przechowywania.

Source: The own study

Źródło: Badania własne

International standards state that, fermented products claiming health benefits must contain a minimum of 10^6 CFU/mL viable probiotic cells per gram of product at the time of the consumption [8]. In our study, the viable cell counts of potential probiotic strain *Lactobacillus casei* O12 in fermented tomato juice with 3% addition of sea buckthorn juice reached this recommendation, which can have beneficial effect on consumer's health.

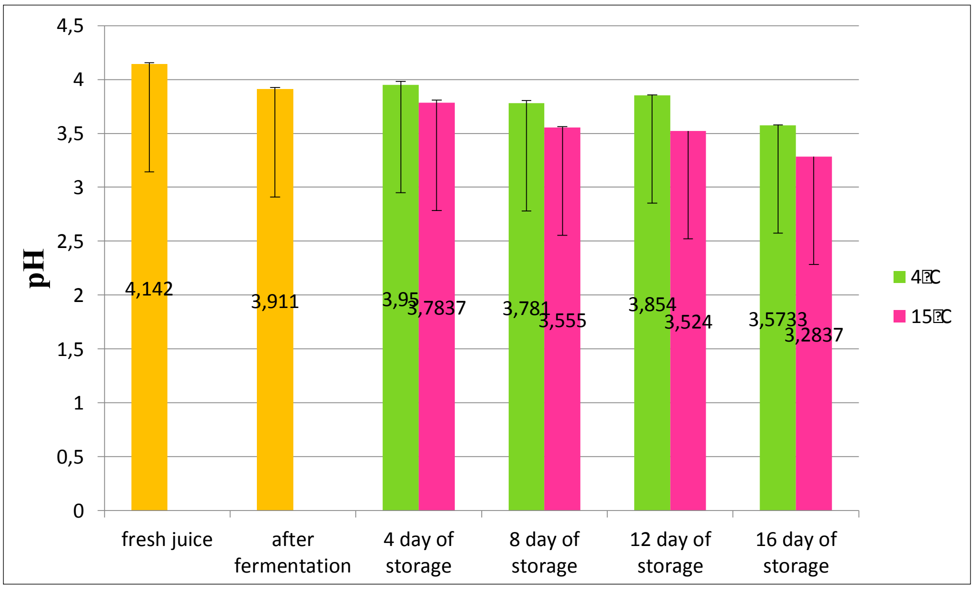

Fig. 2. Changes in pH of fermented tomato juice with 3% addition of sea buckthorn during storage.

Rys. 2. Zmiany pH fermentowanego soku pomidorowego z 3% dodatkiem soku z owoców rokitnika podczas przechowywania.

Source: The own study

Źródło: Badania własne

A low pH is one of the most important factors which can negative effects on viability microorganisms [23]. In our study, the pH value of fresh tomato juice with 3% addition of sea buckthorn juice was 4.142 and lowered to 3.911 after 18 hours of fermentation, which proves the active fermentation carried out by examined potential probiotic strain *Lactobacillus casei* O12. During the storage of juice at both temperatures, there was a decrease in pH. This fact can be attributed to the increase in lactic acid, ethanol and carbon dioxide as a result of lactic acid fermentation [7, 22]. The statistical analysis showed that the storage time and the temperature had a statistically significant effect on changes in the pH values of juices stored at both temperatures ($p<0.05$). In the juice that was stored at the higher temperature, i.e. at 15°C, showed more rapid drop in pH. The pH value of this juice decreased to 3.284 after 16 days of storage. At the same time, more intensive growth of *Lactobacillus* casei O12 cells was observed in the juice stored

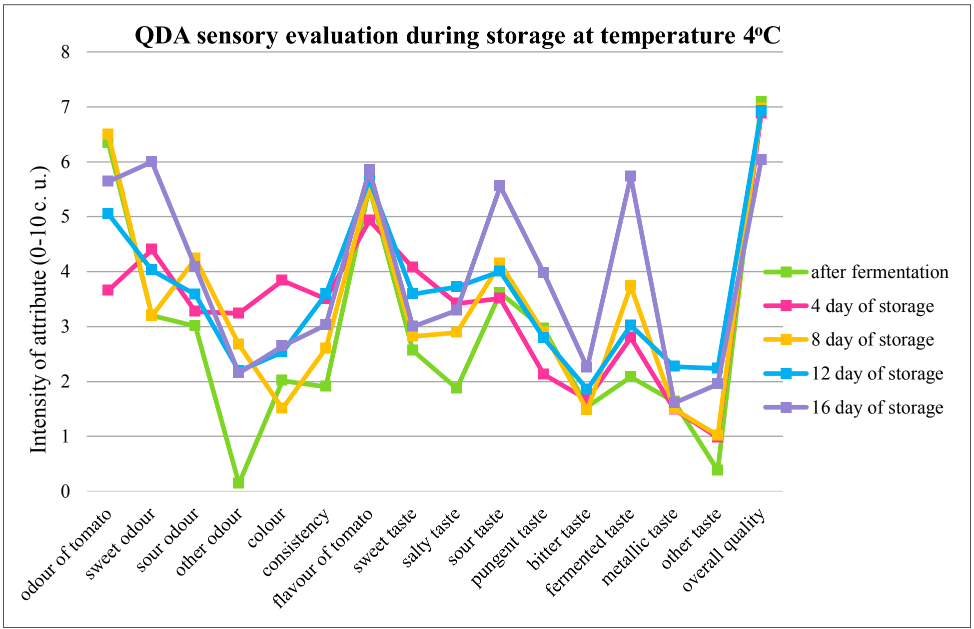

Fig. 3. Characteristic of the sensory quality of tomato juice with 3% addition of sea buckthorn during storage in 4°C.

Rys. 3. Charakterystyka jakości sensorycznej fermentowanego soku pomidorowego z 3% dodatkiem soku z owoców rokitnika podczas przechowywania w 4°C.

Source: The own study

Źródło: Badania własne

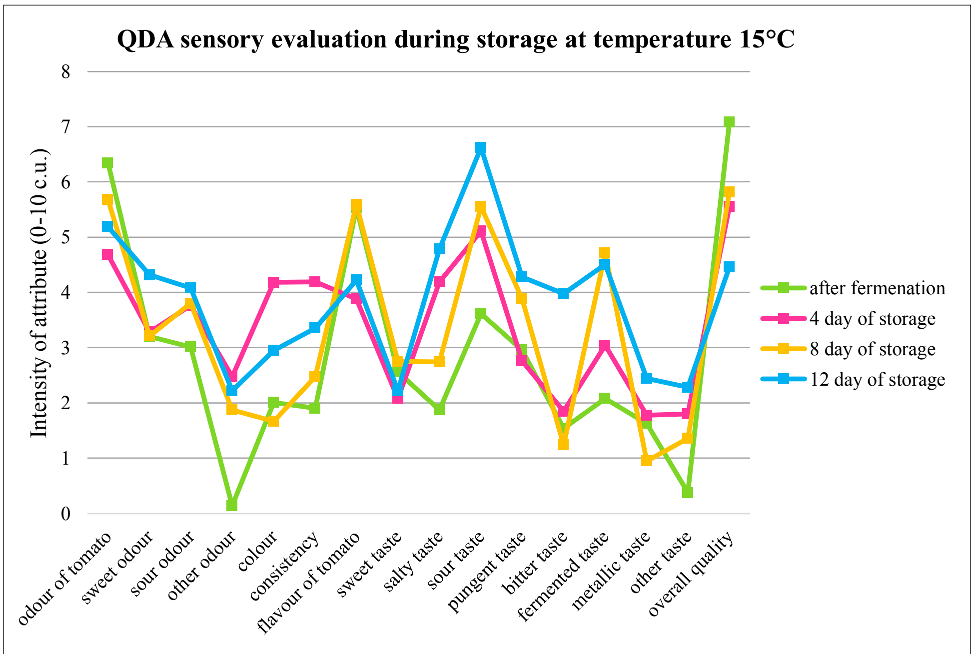

Fig. 4. Characteristic of the sensory quality of tomato juice with 3% addition of sea buckthorn during storage in 15°C.

Rys. 4. Charakterystyka jakości sensorycznej fermentowanego soku pomidorowego z 3% dodatkiem soku z owoców rokitnika podczas przechowywania w 15°C.

Source: The own study

Źródło: Badania własne

at this temperature (Fig. 1). The strain of *Lactobacillus* casei O12 used in the study was able to survive to the end of storage despite the low pH of the juice.

Sensory quality is very important for consumers. The fermentation of some plant juices could improve their sensory quality [9, 13, 19]. In our study the fermented tomato juice with 3% addition of sea buckthorn was characterised by the high overall quality after fermentation (above 7 c.u.).

The average results of intensity of chosen attributes characterising the tomato juice with 3% addition of sea buckthorn are presented in Fig 3 and Fig 4. The intensity of the sour taste, fermented taste, and other taste increased in juices stored at the temperature 4ºC and 15ºC. Changes of sweet odour intensity during storage at both temperatures were insignificant (Fig 3 and Fig 4.).

The overall quality of the juice stored at 4ºC was high until the end of the storage period (Fig. 5.). Changes in the overall quality of the juice stored at this temperature were statistically insignificant. The overall quality of the juice stored at 15ºC decreased significantly from 7.1 c.u. to 5.6 c.u. after 4 days of storage (Fig.5.). After 12 days of storage the overall quality of this juice decreased to 4,5 c.u. below the level accepted by consumers. The decrease of overall quality of the juice stored at 15ºC may be due to a significant increase in the intensity of negative attributes: sour taste and fermented taste. The significant ($p < 0,05$) increase in the intensity of bitter taste from 1.5 c.u. to 3.9 c.u. was also observed between the 8th and 12th day of storage of this juice (Fig. 4.). In the juice stored at 4ºC observed changes in the intensity of bitter taste were not statistically significant. The intensity of fermented taste of juice stored at 15ºC significantly increased after 8 days of storage, while the fermented taste of juice stored at 4ºC increased in the last day of storage (Fig. 3., Fig. 4.). The results of the sensory evaluation indicate, that the tomato juice with 3% addition of sea buckthorn juice can be stored at 4ºC for 16 days and 8 days at 15ºC.

CONCLUSIONS

Tomato juice with 3% addition of sea buckthorn juice is a good raw material for the growth of potentially probiotic bacteria *Lactobacillus casei* O12. The bacteria cells of the tested strain survived during 16 days of storage under the low pH of the juice (below 3.6). The cell count of *Lactobacillus casei* O12 until the end of the storage at 4ºC and 15ºC remained at a high level (9.3 and 9.4 log CFU/mL respectively) allowing the product to be considered probiotic according to FAO/WHO requirements.

After fermentation, the overall quality of tomato juice with 3% addition of sea buckthorn juice with using potentially probiotic *Lactobacillus casei* O12 was high (above 7 c.u.). The results of the sensory evaluation indicate, that tomato juice with 3% addition of sea buckthorn juice can be stored at 4ºC for 16 days and 8 days at 15ºC.

The tomato juice with 3% addition of sea buckthorn juice fermented with *Lactobacillus casei* O12 can be favourable as a probiotic juice, however the consumption effects should be investigated in future studies.

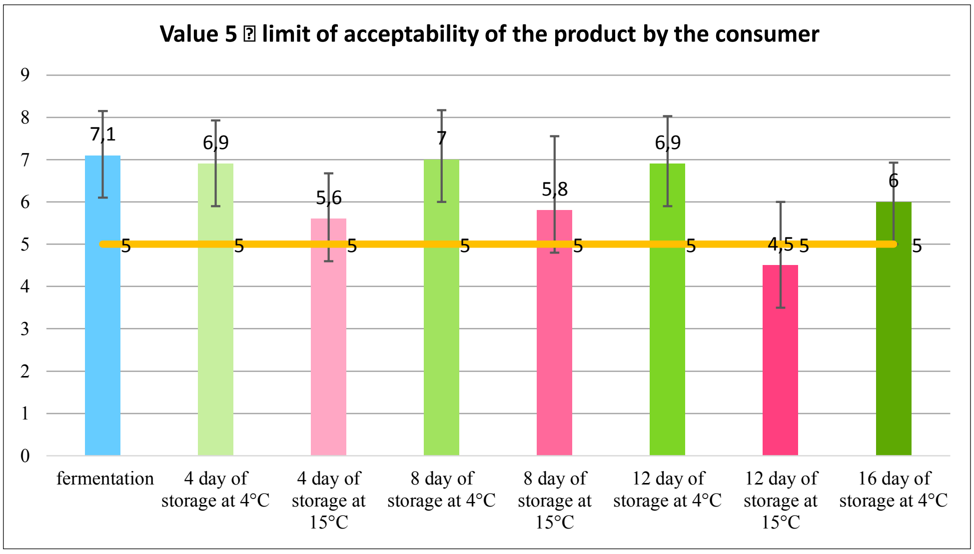

Fig 5. Changes in the overall quality of fermented tomato juice with 3% addition of sea buckthorn during storage.

Rys. 5. Zmiany jakości ogólnej fermentowanego soku pomidorowego z 3% dodatkiem soku z owoców rokitnika podczas przechowywania.

Source: The own study

Źródło: Badania własne

WNIOSKI

Sok pomidorowy z 3% dodatkiem soku z owoców rokitnika jest dobrym surowcem do wzrostu potencjalnie probiotycznych bakterii *Lactobacillus casei* O12. Stwierdzono obecność żywych komórek badanego szczepu po 16 dniach przechowywania soku o niskim pH (poniżej 3,6). Liczba komórek *Lactobacillus casei* O12 do końca przechowywania w 4ºC i 15ºC utrzymywała się na wysokim poziomie (odpowiednio 9,3 i 9,4 log jtk/ml) i produkt spełniał wymagania FAO/WHO odnośnie minimalnej liczby komórek dla produktów probiotycznych. Po fermentacji jakość ogólna soku pomidorowego z 3% dodatkiem soku z owoców rokitnika z użyciem potencjalnie probiotycznego szczepu *Lactobacillus casei* O12 była wysoka (powyżej 7 j.u.). Wyniki oceny sensorycznej wskazują, że sok pomidorowy z 3% dodatkiem soku z owoców rokitnika fermentowany *Lactobacillus casei* O12 może być atrakcyjnym sokiem probiotycznym, jednak niezbędne są dalsze badania odnośnie jego właściwości prozdrowotnych.

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