Dr inż. Barbara SIONEK
Institute of Human Nutrition Sciences, Warsaw University of Life Sciences (WULS, Poland)
Instytut Nauk o Żywieniu Człowieka, Szkoła Główna Gospodarstwa Wiejskiego w Warszawie (SGGW, Polska)

EVALUATION OF THE ADDITION OF PREBIOTICS ON THE QUALITY OF PROBIOTIC COCONUT DESSERTS®

Ocena wpływu dodatku prebiotyków na jakość probiotycznych deserów kokosowych®

Key words: prebiotics, probiotics, synbiotics, functional foods, *Lactobacillus, fermented foods.*

The aim of this study presented in the article was to develop a synbiotic coconut dessert with the satisfactory sensory quality without sugar, with the addition of probiotic bacteria and prebiotics. A selected strain of probiotic bacteria and the following prebiotics were added to the coconut desserts: inulin, maltodextrin, guar gum, β-glucan. Coconut drink was fermented at 37°C for 24 hours and stored at 4°C for 7 days. The dessert with Lactobacillus plantarum 299v had the best sensory quality and was selected for the study with using prebiotics. In the dessert with the addition of prebiotics, the survivability of the Lactobacillus plantarum 299v strain was high (>8 log CFU/mL) both after fermentation and during storage. The overall quality of the coconut dessert with Lactobacillus plantarum 299v and prebiotics was high after fermentation and during storage. At the end of the storage period, the highest overall quality has a coconut dessert with inulin and control sample. Coconut desserts have the required minimum number of cells of the Lactobacillus plantarum 299v strain and they can be considered as probiotic products.

Słowa kluczowe: prebiotyki, probiotyki, synbiotyki, żywność funkcjonalna, *Lactobacillus, żywność fermentowana.*

Celem pracy przedstawionej w artykule było opracowanie synbiotycznego deseru kokosowego bez dodatku cukru o zadowalającej jakości sensorycznej, z dodatkiem bakterii probiotycznych i prebiotyków. Do deseru kokosowego dodano wyselekcjonowany szczep bakterii probiotycznych oraz następujące związki prebiotyczne: inulinę, maltodekstrynę, gume guar, β-glukan. Napój kokosowy fermentowany był w temperaturze 37°C przez 24 godziny i przechowywany w 4°C przez 7 dni. Jako kulturę startową zastosowano szczepy bakterii probiotycznych i potencjalnie probiotycznych. Deser ze szczepem Lactobacillus plantarum 299v miał najlepszą jakość sensoryczną i został wybrany do badania z zastosowaniem prebiotyków. W deserze z dodatkiem prebiotyków przeżywalność szczepu Lactobacillus plantarum 299v była wysoka (>8 log jtk/mL) zarówno po fermentacji, jak i podczas przechowywania. Ogólna jakość deseru kokosowego z Lactobacillus plantarum 299v i prebiotykami była wysoka po fermentacji i podczas przechowywania. Pod koniec okresu przechowywania najwyższą ogólną jakość miał deser kokosowy z inuliną i próba kontrolna. Desery kokosowe zawierały wymaganą minimalną liczbę komórek Lactobacillus plantarum 299v, tym samym mogą być uznane za produkty probiotyczne.

INTRODUCTION

The interest in food that has a beneficial effect on health is a trend observed all over the world. Consumers increasingly make informed choices: they avoid food ingredients that contribute to diseases, such as animal fats, simple sugars, chemical preservatives and colorants, choose low-calorie foods, look for tasty and nutritious products. Functional foods becoming more popular. Positive opinions about the health-promoting effect of functional foods, in particular on functioning of the digestive and immune systems and the possible inhibition of the development of many diseases have been confirmed by many scientific studies and recommendations of authoritative institutions and agencies as FAO/WHO, EFSA, FDA, ESPHAGAN [6, 8, 18, 23]. It is believed that prebiotics may have health benefits. Prebiotics by increasing the number of commensal bacteria improve the balance in the human intestinal ecosystem [7, 25]. They have an impact on endocrine function of gastrointestinal tract, modulate gastrointestinal peptides, enhance immune system [1, 3, 4, 22]. More investigations are necessary to better understanding of mechanism involved and to prove the health effects associated with the consumption of prebiotics [14, 19]. Substances that are considered prebiotics are added to various food products, such as dairy products, plant-based drinks, health drinks, infant formulas and meat products [9]. Inulin and oligofructose are most common prebiotics on the market used in food production [13, 15].

Corresponding author – Adres do korespondencji: Barbara Sionek, Institute of Human Nutrition Sciences, Warsaw University of Life Sciences (WULS), Department of Food Gastronomy and Food Hygiene, Nowoursynowska Str. 159c, 02-776 Warsaw, Poland, e-mail: barbara_sionek@sggw.edu.pl

Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host [8]. The growing interest in probiotics is primarily related to their scientifically proven beneficial influence on the functioning of the digestive and immune systems and the possible inhibition of many diseases. Development of new functional foods containing probiotics and prebiotics is of great interest, because of the significant health benefits [2, 10]. The addition of probiotic and potentially probiotic microorganisms and/or prebiotics is positively perceived by consumers and is an incentive for food producers to search for new food products that meet the criteria of functional food. The combination of the addition of probiotic microorganisms and prebiotics in one food product results in a synbiotic product that seems to have an optimal effect on consumer health [17]. Gibson and Roberfroid (1995) first defined synbiotic as a mixture of probiotics and prebiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and/or by activating the metabolism of one or a limited number of health-promoting bacteria, and thus improving host welfare [11]. Choosing the right probiotic-prebiotic pair for a given food product is crucial and should maintain proper viability of the probiotic microorganisms during the storage period [24].

In addition to the health-promoting effect of synbiotics, a prerequisite for the development of such a product is to maintain or improve its sensory quality. In accordance with the above-mentioned preferences, the aim of the study was to develop a synbiotic coconut desserts without sugar and with the addition of probiotic bacteria and prebiotics with the satisfactory sensory quality.

MATERIAL AND METHODS

Material. The probiotic bacterial strains: *Lactobacillus plantarum* 299v*, Lactobacillus acidophilus* LA5*, Lactobacillus casei* 431 came from the collection of the Institute of Fermentation Technology and Microbiology of the Lodz University of Technology; potentially probiotic: *Lactobacillus casei* O12 *and Lactobacillus johnsonii* K4, came from the collection of Institute of Human Nutrition Sciences, Warsaw University of Life Sciences and were used as a starter culture. *Lb. casei* 012*, Lb. johnsonii* K4 were isolated from fermented cabbage and cucumber and their selected probiotic properties were confirmed in the previous studied [30].

Inulin, maltodextrin (China, distribution PPH Apimar, Poland), β-glukan (Medicaline Aliness, Poland) and guar gum (India, distribution PPH Apimar, Poland) were used as prebiotic components in the experiment. Desserts were made on the basis of a coconut drink without sugar (manufacturer Alpro, Wevelgem, Belgium) purchased at the local store.

Probiotic starter culture preparation. Pure LAB strains were stored in -80°C and revitalised before use. 5 mL of MRS broth (Biokar Diagnostics, France) was inoculated with the bacterial strain then the suspension was incubated at 37°C for 24h.Then 1 mL of culture was added to 9 mL of MRS broth and incubated again at 37°C for 24h.The 24-hour bacterial culture was added to the drink, after the broth was centrifuged (10 000g/5min) and replaced with the coconut drink. The number of examined bacteria cells in the starter culture used to inoculate a coconut drink was 9 log CFU/mL.

Fermented coconut desserts preparation. The study was divided into two stages. In the first stage of the research, the most suitable strain for the fermentation of the drink was selected, and sensory evaluation was used as a selection criterion. The second part of the study included the evaluation of the effect of addition of prebiotics on the growth of the bacteria strain selected in the first stage of experiment, after fermentation and during refrigerated storage. For that purpose, the coconut drink was supplemented with selected starter strain and independently with the addition of 1% (w/v) of the following prebiotics: inulin, maltodextrin, guar gum, β-glukan. The control sample without prebiotic added was also prepared. The drink was fermented at 37°C for 24 hours and stored in 4°C.

The microbiological analysis. The number of bacteria cells were determined using the deep plate method, from three subsequent dilutions and plates on a selective MRS agar (Biocar, Diagnostics, France). The plates were incubated at 37°C for 48 hours.

Measurement of pH. The pH values were measured using of pH meter Elmetron CP 501 (Elmetron sp. j., Poland) potentiometric method at room temperature, just after fermentation and after 3 and 7 days of storage. The pH-meter was equipped with automatic temperature compensation and combined electrode at two points (pH=4 i pH=7).

Sensory analysis. The sensory scaling and ranking method were applied for the selection of the strain [26]. To determine sensory quality changes of the desserts after fermentation and during storage the Quantitative Descriptive Analysis (QDA) was used [28]. As a result of the selection 14 quality descriptors were chosen including four descriptors of smell, seven descriptors of ftaste, consistency, colour and overall quality. The intensity of descriptors was marked on a unstructured linear scale [0 – 10 c.u.]. The panelists possessed the necessary skills to describe the sensory attributes of different samples. The trained panel were extensively and formally tested before being selected, according to the [27]. The assessment was carried out in a room free from foreign odors with natural light and minimized noise level at room temperature.

Statistical analysis were performed using statistica software (Statistica 13.3.,StatSoft, Poland). Analysis of variance (ANOVA) was used to assess the significance of the effects ($P < 0.05$). The difference between means was detected by the Tukey's test.

RESULT AND DISCUSSION

Selection of a Lactobacillus probiotic and potentially probiotic strain for the production of coconut dessert

In the study on the selection of the strain for fermentation the sensory evaluation was used as a way to assess the consumer preference of the product. Figure 1 shows the average results of sensory evaluation among individual samples of fermented coconut desserts with the use of selected strains of probiotic and potentially probiotic bacteria. The

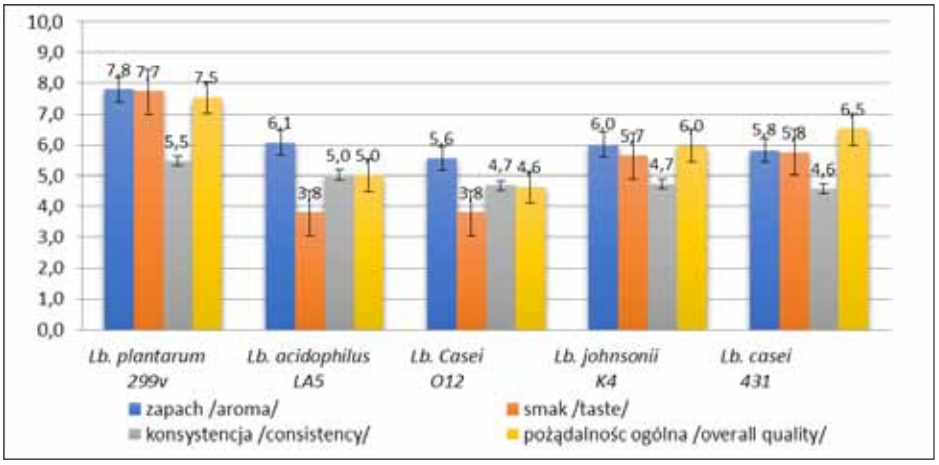


Fig. 1. Results of ratings of attributes of sensory quality of fermented coconut dessert depending on the strain of probiotic and potentially probiotic bacteria used - scaling method.

Rys. 1. Wyniki pożądalności wyróżników jakości sensorycznej fermentowanego deseru kokosowego w zależności od użytego szczepu bakterii probiotycznych i potencjalnie probiotycznych - metoda skalowania.

Source: The own study

Źródło: Badania własne

product inoculated with the strain *Lactobacillus plantarum* 299v achieved statistically significantly higher ($p>0.05$) desirability of taste, smell, overall quality compared to other samples. The taste was the descriptor that the most differentiated the samples. The results shown that the dessert with the addition of the strain *Lactobacillus plantarum* 299v characterised of the highest taste desirability (7.7 c.u.), while desserts with the *Lactobacillus casei* O12 and *Lactobacillus acidophilus* LA5 added, were the lowest taste desirability (3.8 c.u.) (fig.1.). There were no significant differences in the taste desirability for desserts with the addition of strains *Lactobacillus johnsonii* K4 and *Lactobacillus casei* 431 (5.7 c.u. and 5.8 c.u. respectively).

In the ranking method, which forces the order of the samples to be marked (fig. 2), the highest desire of the product with the *Lactobacillus plantarum* 299v strain was also found (the lowest value of the average rank, equal to 1.67). Differences in the sensory quality of desserts using the tested strains may be related to the ability of a given strain to metabolize nutrients and to produce compounds affecting smell and taste. Zielińska (2005) also found differences in the sensory quality of fermented (9 h/32°C) soy drinks using 10 strains of *Lactobacillus* [29].

The results obtained by scaling and ranking method indicated that the use of *Lb. plantarum* 299v strain allowed to obtain the best sensory quality of fermented drink. On this basis, it was decided to use this strain for further research.

Assessment of the change in the number of bacteria and pH of the coconut dessert after fermentation and during storage

Probiotic survival was evaluated in the coconut deserts after fermentation and at 3 and 7 days of storage. Survival of *Lactobacillus plantarum* 299v strain was high (cell counts was approximately 8 log CFU/mL) both after fermentation and during storage (fig. 3). As a result of the analysis, it was found that time had a significant ($p<0.05$) effect on the change in the number of cells of *Lb. plantarum* 299v in fermented samples. In coconut desserts with inulin and maltodextrin, a gradual increase in the number of cells of the probiotic strain was observed until the last day of storage studies.

Despite of increase in the number of bacteria, there were no significant changes in the pH in the dessert with inulin after fermentation and during storage (pH: 4.230, 4.350, 4.290 respectively) (fig. 4). In the dessert with β-glucan and a control sample, the number of probiotic bacteria cells on the 3rd day of storage significantly decreased, compared to the number of cells after fermentation. In desserts with maltodextrin, guar gum and inulin, the number of *Lb. plantarum* 299v that day was higher than after fermentation. In the study of Donkor et al. (2007) the addition of 2% inulin,1% of raffinose and 1% of glucose in fermented soya drink had a positive impact on the viability of *Lactobacillus acidophilus* L10, *Bifidobacterium animalis* B94 and *Lactobacillus casei* L26 [5].

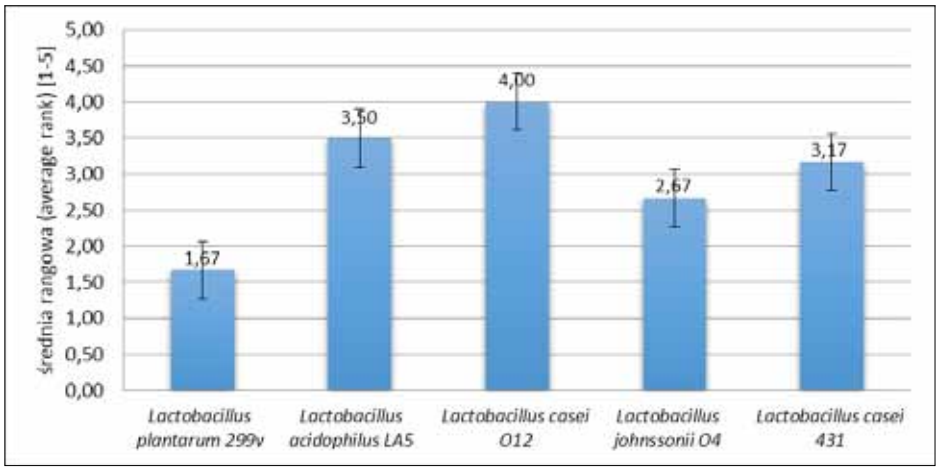
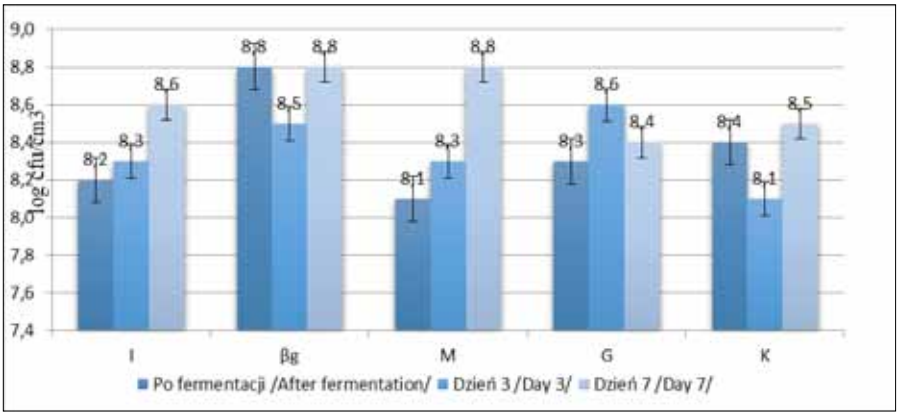


Fig. 2. Results of acceptability assessment of fermented coconut drink samples depending on the strain of probiotic and potentially probiotic bacteria used (ranking method, 1 – the best, 5 – the worse, $p < 0.05$).

Rys. 2. Wyniki oceny pożądalności fermentowanego napoju kokosowego w zależności od użytego szczepu bakterii probiotycznych i potencjalnie probiotycznych (metoda szeregowania, 1 – najlepszy, 5 – najgorszy, $p < 0,05$).

Source: The own study

Źródło: Badania własne



Explanatory notes: I – inulin, Bg - β-glukan, M – maltodextrin, G – guar gum, K – control sample

Objaśnienia: I - inulina, Bg – β-glukan, M – maltodekstryna, G – guma guar, K – próba kontrolna)

Fig. 3. The number of *Lactobacillus plantarum* 299v in the coconut desserts with 1% addition of prebiotic after fermentation (24 h/37°C) and after 3 and 7 days of storage in 4°C.

Rys. 3. Liczba komórek *Lactobacillus plantarum* 299v w deserach kokosowych z 1% dodatkiem prebiotyku po fermentacji (24 godz./37°C) oraz po 3 i 7 dniach przechowywania w 4°C.

Source: The own study

Źródło: Badania własne

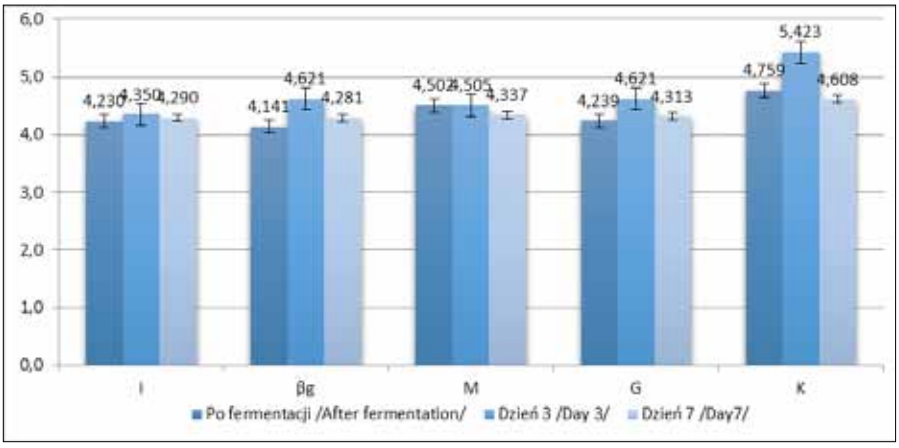


Fig. 4. pH values of coconut desserts made with the use of *Lactobacillus plantarum* 299v strain and with 1% addition of prebiotic after 24 h fermentation and after 3 and 7 days of storage in 4°C (explanatory notes as at fig. 3).

Rys. 4. Wartości pH w deserach kokosowych z użyciem szczepu *Lactobacillus plantarum* 299v i 1% dodatkiem prebiotyku po 24h fermentacji oraz po 3 i 7 dniach przechowywania w 4°C(objaśnienia jak na rys. 3).

Source: The own study

Źródło: Badania własne

The selected probiotic strains should maintain their viability during the entire shelf-life of the product. Many factors may affect the viability of bacteria selected strains, including added ingredients, pH, the presence of hydrogen peroxide and dissolved oxygen, the concentration of lactic acid, storage temperature [20, 21]. A factor that can directly affect the survival of bacterial strains is pH. According to Zielińska et al. (2019), lactic acid bacteria can survive in an environment with a pH of 4.5 to 7.0 and this is an individual feature of a specific genus, species or strain [30]. During storage, the pH of all coconut desserts with the addition of prebiotics was low, below 4.65 (fig. 6). The *Lb. plantarum* 299v was able to survive at low pH until the end of storage.

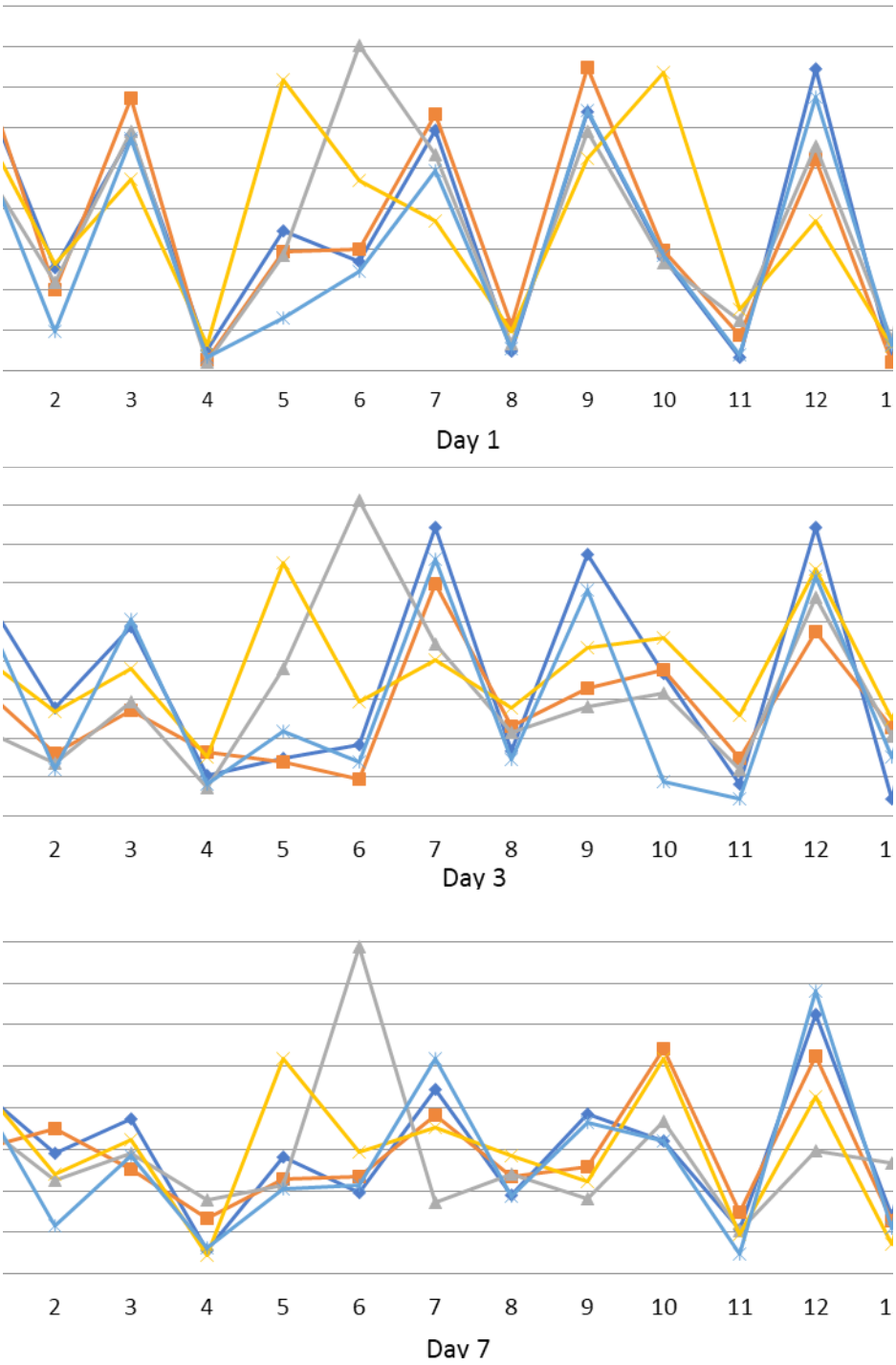
On the last day, the number of *Lb. plantarum* 299v in all tested variants of coconut desserts was high (from 8.4 log CFU/mL for dessert with guar gum to 8.8 for dessert with β-glucan), which indicates a good adaptation of the strains to environmental conditions (low pH) and the appropriate contents of nutrients in the coconut desserts. According to the FAO/WHO guidelines for both primary and secondary microflora (probiotic microflora), the number of live cells of lactic acid bacteria on the last day of shelf life of the product should not be lower than 10^6 CFU/mL [8]. The minimum number of probiotic bacteria requirement has been fullfilled.

The evaluation of the sensory quality of desserts with the addition of prebiotics

The average results of the profiling (QDA) analysis of the fermented desserts with prebiotics added are presented at fig.5.

The coconut dessert with the addition of inulin was characterized by high intensity of sweet taste both after fermentation and during storage, which in the case of a sugar-free product was positively perceived by assessors. Additionally, the high intensity of coconut taste, characteristic for coconut dessert and the milk taste and milk smell, possibly contributed to maintaining the high overall quality of this dessert throughout the study. The sensory characteristic of the product are influenced by the concentration of the prebiotic. In the study of Guven et al. (2005) it was found, that the addition of 1% inulin guaranteed an increase of the positive sensory characteristics of the product, and its greater amount already had a negative effect on the overall acceptability of yogurts and their consistency [12].

After fermentation, the most thick consistency had a dessert with guar gum, which is often used in food production as a stabilizer to give the products the desired texture [16]. β-glucan not only influenced the change of colour to a more yellow one, but also significantly increased intensity of the sour taste of a dessert, to a value of over 7 c.u. (fig. 5a). Other products, in the assessment of the intensity of sour taste reached values below 3 c.u., including dessert with maltodextrin,



Attributes/Wyróżniki: 1. sweet smell/zapach słodki, 2. sour s./zapach kwaśny, 3.milk s./zapach mleczny,4. irritating s/z. drażniący, 5. colour/barwa 6.konsystencja/consistency, 7. sweet taste/ smak słodki, 8. salty t./smak słony, 9. milk t./smak mleczny, 10. sour t./smak kwaśny,11. bitter t./smak gorzki,12. coconut t./smak kokosowy, 13. other t./smak obcy,14. overall quality/ jakość ogólna

Fig. 5a, b, c. Results of intensity of sensory attributes [c.u.] assessment of coconut prebiotic desserts - QDA method: a) after fermentation 24h/37°C b) after 3 days of storage at 4°C c) after 7 days of storage at 4°C (explanatory as at fig. 3).

Rys. 5a, b, c. Wyniki oceny intensywności wyróżników sensorycznych [j.u.] deserów kokosowych - metoda QDA: a) po fermentacji 24 godz./37°C b) po przechowywaniu 3 dni w 4°C c) po przechowywaniu 7 dni w 4°C (objaśnienia jak na rys. 3).

Source: The own study

Źródło: Badania własne

which at the same time achieved high intensity of milk taste (approximately 7.5 c.u.) and sweet taste (below 6 c.u.) (fig. 5a). Dessert with β-glucan was characterized by the intensity of the descriptors: coconut taste, sweet taste and milk smell below 5 c.u. In addition, the overall quality of this product was assessed as the lowest. Bitter and other taste, as negative quality descriptors were at low intensity in all studied products, while positive descriptors: coconut, milk and sweet taste as well as sweet and milky smell were at a high level (except for dessert with β-glucan), which could have a direct impact on the high overall quality of these products.

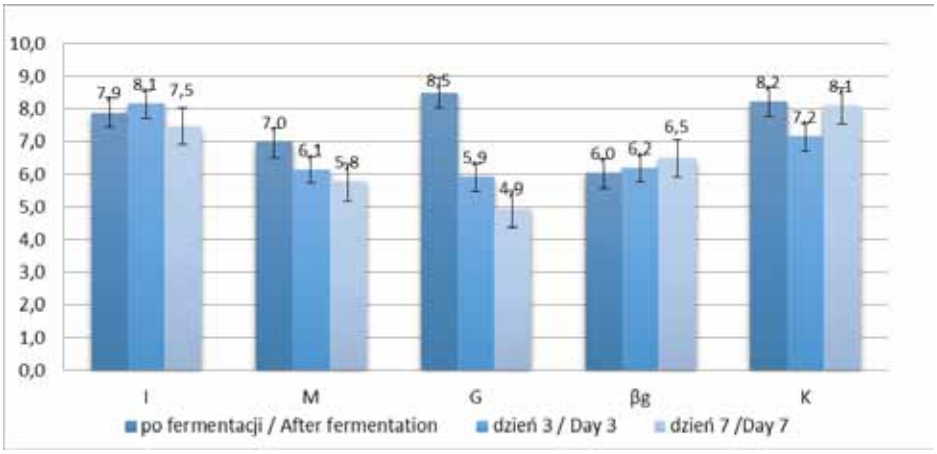
During storage, all desserts lost their sweet smell, which was less noticeable by assessors. For example, the intensity of the descriptor: sweet smell, for a dessert with inulin, decreased from over 7 c.u. (after fermentation) to approx. 6 c.u. and 5 c.u. on the 3rd and 7th day respectively (fig. 5b, c). During the storage, an increase in the perceptibility of smell and sour taste was noticed, which may be related to the increasing amount of lactic acid, formed as a result of lactic acid fermentation carried out by probiotic bacteria. On the seventh day, a product containing β-glucan obtained low intensity of positive descriptors ie.: sweet, milk and coconut taste, which could adversely affect the overall quality. This variant of the dessert was evaluated be the least desirable, characterised by a significant reduction in the intensity of milk smell and milk taste and an increase in the intensity of sour, other, salty and irritating taste. All the above-mentioned changes had an impact on the overall quality of the final product and caused a decrease in its attractiveness.

Sensory analysis showed that the addition of guar gum caused an increase in thick consistency of the coconut dessert, which was observed during the entire storage period. Dessert with the addition of guar gum on the 7th day of storage was characterised by the lowest descriptors for sweet, milk and coconut taste. These characteristics may have adversely affected the overall quality and, together with the decrease in the intensity of sweet taste, contributed

to the lowest overall quality of the studied dessert on the 7th day of storage (fig. 5c).

The addition of maltodextrin and β-glucan in the coconut desserts contributed to the highest intensity of sour taste both after fermentation and during storage, which may lower the overall quality (fig. 5a, b, c).

The overall sensory quality of desserts with *Lactobacillus plantarum* 299v and prebiotics was high after fermentation and during storage. After fermentation, the highest overall quality had the dessert with guar gum and the control sample (8.5 and 8.2 c.u. respectively) (fig. 6). The overall quality of the dessert with guar gum decreased from 8.5 c.u. after fermentation to 4.9 c.u. on the last day of storage, and the product was no longer sensory accepted. The overall quality of the dessert with β-glucan, inulin and maltodextrin was high during the entire storage time.



(Oznaczenia odpowiednio: I – inulina, M – maltodekstryna, G – guma guar, Bg – β-glukan, K – próba kontrolna)

Fig. 6. Comparison of the overall quality of coconut dessert samples with the addition of prebiotic (1%) and *Lactobacillus plantarum* 299v strain after 24 h of fermentation and 3 and 7 days of storage (QDA method).

Rys. 6. Porównanie ogólnej jakości próbek deseru kokosowego z dodatkiem prebiotyku (1%) i szczepem *Lactobacillus plantarum* 299v po 24 godz. fermentacji oraz 3 i 7 dniu przechowywania (metoda QDA).

Source: The own study

Źródło: Badania własne

CONCLUSIONS

1. The experiment allowed to selected from 5 probiotic and potentially probiotic strains – *Lactobacillus plantarum* 299v strain, which made it possible to obtain a coconut dessert with the highest sensory quality.
2. The number of viable cell counts of *Lactobacillus plantarum* 299v in the studied coconut desserts with the addition of various prebiotics: inulin, maltodextrin, β-glucan and guar gum remained at a high level during the storage period.
3. The obtained coconut desserts had a minimum number of cells of the *Lactobacillus plantarum* 299v and thus could be determined as probiotic products.
4. The sensory quality of the tested coconut desserts with the addition of *Lactobacillus plantarum* 299v strain was high both after fermentation and at the end of the storage period (7 days); only the overall quality of the product with 1% addition of guar gum on the last day of storage was 4.9 c.u. and the product was no longer sensory accepted.

WNIOSKI

1. Przeprowadzone doświadczenie pozwoliło na wybranie spośród 5 szczepów probiotycznych i potencjalnie probiotycznych – szczepu *Lactobacillus plantarum* 299v, który umożliwił uzyskanie deseru kokosowego o najwyższych notach w ocenie sensorycznej.
2. Liczba komórek szczepu *Lactobacillus plantarum* 299v w badanych deserach kokosowych z dodatkiem różnych prebiotyków: inuliny, maltodekstryny, β-glukanu oraz gumy guar utrzymywała się na wysokim poziomie przez cały okres przechowywania.
3. Uzyskane desery charakteryzowały się wymaganą, minimalną liczbą komórek szczepu *Lactobacillus plantarum* 299v i tym samym mogły zostać uznane za produkty probiotyczne.
4. Jakość sensoryczna badanych deserów kokosowych z dodatkiem szczepu *Lactobacillus plantarum* 299v była wysoka zarówno po fermentacji jak i do końca okresu przechowywania (7 dni); jedynie jakość ogólna produktu z 1% dodatkiem gumy guar ostatniego dnia przechowywania wynosiła 4,9 j.u. i produkt nie był już akceptowany pod względem jakości sensorycznej.

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