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FERMENTATION OF PLANT-BASED BEVERAGES USING LACTIC ACID BACTERIA – A REVIEW®

Fermentacja napojów roślinnych z wykorzystaniem bakterii kwasu mlekowego – przegląd literatury®

Key words: milk substitutes, fermentation, viability of bacteria, probiotics, food trends, plant-based beverages.

The number of people following plant-based diets is increasing tremendously. One of the most popular plant replacements for animal products is plant-based beverages, which are one of the food groups that are irreplaceable in the vegan food industry. With the growing popularity of plant-based diets, the demand for fermented plant-based beverages is also growing. This article provides a brief overview of the fermentation of plant-based beverages using lactic acid bacteria (LAB). The general characteristics of plant-based beverages were presented and the survival of LAB in different types of plant-based milk substitutes was analyzed. The biggest challenge facing producers of fermented plant-based beverages is to ensure a sufficiently high survival rate of probiotic bacteria, which are responsible for causing the health effect. This review showed that the presented milk substitutes made from cereals, pseudocereals, legumes, seeds, nuts, and other raw materials constitute a suitable matrix for the fermentation. Despite many studies and experiments, the topic is still relevant, with many scientific reports finding a place in top trade journals.

Słowa kluczowe: substytuty mleka, fermentacja, przeżywalność bakterii, probiotyki, trendy w żywności, napoje roślinne.

Liczba osób stosujących diety roślinne rośnie w zaskakująco szybkim tempie. Jednym z najpopularniejszych zamienników roślinnych produktów odzwierzęcych są napoje roślinne, które stanowią jedną z grup żywności niezastąpionych w wegańskim przemyśle spożywczym. Wraz z rosnącą popularnością diet roślinnych rośnie również zapotrzebowanie na fermentowane napoje roślinne. Niniejszy artykuł przedstawia krótki przegląd literatury dotyczącej fermentacji napojów roślinnych z wykorzystaniem bakterii kwasu mlekowego (LAB). Przedstawiono ogólną charakterystykę napojów roślinnych oraz przeanalizowano przeżywalność LAB w różnych rodzajach roślinnych substytutów mleka. Największym wyzwaniem stojącym przed producentami fermentowanych napojów roślinnych jest zapewnienie odpowiednio wysokiej przeżywalności bakterii probiotycznych, odpowiedzialnych za wywoływanie efektu zdrowotnego. Przegląd ten wykazał, że przedstawione napoje roślinne, wytworzone ze zbóż, pseudozbóż, strączków, nasion, orzechów i innych surowców, stanowią odpowiednią matrycę do procesu fermentacji. Pomimo wielu badań i eksperymentów, temat ten jest jednak nadal aktualny, a wiele doniesień naukowych znajduje swoje miejsce w czołowych czasopismach branżowych.

INTRODUCTION

Over the past decade, research is mainly focused on developing products targeting the changing demands of the consumer, by creating newer alternatives to health foods. Researchers focus on plant foods rich in dietary fibers, minerals, vitamins, and antioxidants. Such products are often classified as functional food, i.e. one that has a documented positive effect on the human body and its consumption may improve health and well-being, as well as reduce the risk of diseases [42, 46].

Plant-based products are often presented as a healthy, sustainable, and animal-welfare-friendly alternative [23]. These factors push the food industry and the global market toward the design, supply, and production of novel plant-based products [43]. Importantly, vegetarianism, veganism and the adoption of a plant-based diet are growing trends, mainly across Western countries. The number of people following plant-based diets is increasing tremendously, according to different vegan societies and consulting companies [1].

Throughout the years, the consumption of plant-based diets has increased considerably, which is the result of consumer decisions related to:

– health and environmental concerns;
– global trend of plant-based diets (vegetarian and vegan);

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- aversion to animal cruelty;
- widespread incidence of lactose intolerance or cow's milk protein allergy;
- flexitarian choice of food consumption;
- desire for a healthy lifestyle
- problems due to diets rich in cholesterol [1, 4, 13, 41].

One of the most popular plant replacements for animal products are plant-based beverages, which are one of the food groups that are irreplaceable in the vegan food industry. They are mainly used as milk substitutes; however these products are also used as an essential ingredient in many vegan food products such as plant-based yogurt, cheese, kefir, butter, ice cream, etc. [4, 44]. The global plant-based beverages market reached an estimated size of US $8.51 billion in 2016 and is forecasted to rise to a CAGR (compound annual growth rate) of 12.5% to triple a market volume of US $24.6 billion in 2025 [23]. Soy products are still dominating this market, but the emergence of alternative products from other plant sources (e.g. other legumes, cereals, nuts) has decreased its share [36].

With the growing popularity of plant-based diets, the demand for fermented plant-based beverages is also growing [3, 31]. Such products should contain lactic acid bacteria (LAB) that are naturally present in fermented milk drinks, including yoghurt, kefir, etc. LABs often exhibit probiotic properties and should show a similar survival in a plant matrix as in dairy products [39, 59]. This review provides a brief overview of fermentation of plant-based beverages using LAB. The general characteristics of plant-based beverages were presented and the survival of LAB in different types of plant-based milk substitutes was analyzed.

CHARACTERISTICS OF PLANT-BASED BEVERAGES

Plant-based beverages have gained immense popularity over the years among consumers avoiding cow's milk. Today, milk substitutes are commercially obtained from a variety of plant-derived ingredients [43]. Types of plant-based beverages obtained from various raw materials are presented in Figure 1. There is no stated definition and classification of plant-based beverages in the literature. They are fluids that results from breakdown (size reduction) of plant material extracted in water and further homogenization of such fluids. The size of particles and the stability of the final product depend on the nature of the raw material, the method used for disintegration and storage conditions [46]. Obtained products are similar in appearance and taste to conventional milk and are used for the same purposes. Derived from the water extraction of plant matrices, plant-based beverages are completely free from animal-based ingredients [23]. Depending on the raw materials, fortification and technology employed in the production processes, large nutritional composition variability, and differences in terms of technological and sensory features have been reported [23, 36, 43].

The production of plant-based drinks varies depending on the raw material used. However, each drink has several of the same production steps. Initially, the raw material is soaked and grounded. The obtained grounded product is extracted with water. The solid material is filtered, and the obtained liquid is supplemented with additional ingredients, mainly thickeners (e.g. sunflower lecithin, gellan gum), antioxidants (e.g. ascorbic acid), sweeteners, sea salt, fortifying substances (e.g. calcium, vitamin A, B2, B1, B12, D2, and E). The product is then homogenized, heat treated and packaged [4, 36, 41].

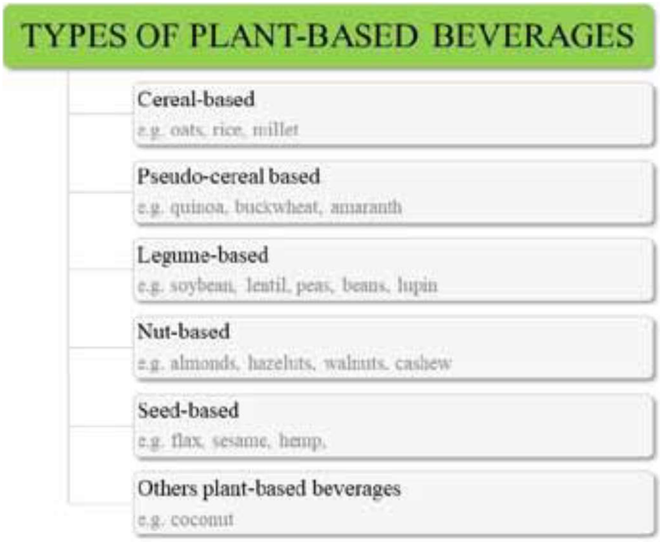

Fig. 1. Types of plant-based beverages.
Rys. 1. Rodzaje napojów roślinnych.
Source: Own study
Źródło: Opracowanie własne

There are great differences in the composition of different types of plant-based beverages. According to Fructuoso et al. [20], the variation in the amount of nutrients in different plant-based beverages was 6–183 kcal/100 mL for energy value; 0.00–22.29 g/100 mL for carbohydrate; 0.06–12.43 g/100 mL for protein; 0.00–19.00 g/100 mL for lipid; 0.00–4.40 g/100 mL for dietary fiber; and 0.00–1252.94 mg/100 mL for calcium [20]. Plant-based beverages are treated as milk substitutes but differ from cow milk in several ways. From the macronutrients point of view, the protein content of milk substitutes is generally lower than in cow's milk, but plant-based milk alternatives are richer in terms of fiber and unsaturated fatty acid content, are cholesterol-free and present a low glycemic index [4, 15, 24]. Plant proteins are generally of a lower nutritional quality compared to animal - derived proteins due to limiting amino acids (lysine in cereals, methionine in legumes) and poor digestibility [36]. Plant-based beverages are sometimes enriched with calcium, vitamin D, and B vitamins, but still many products on the market are not fortified [15, 23].

Plant-based beverages are rich in bioactive compounds, the presence of which has many health-promoting effects. These ingredients include e.g. beta-glucans, phytosterols, isoflavones, fatty acids, and lignans [41]. The presence of bioactive ingredients makes regular consumption of plant-based beverages associated with improved blood glucose and insulin resistance; reducing the risk of cardiovascular disease and diabetes; reducing the occurrence of some cancer and osteoporosis; supporting the immune system; as well as reducing the blood cholesterol level [4, 36, 41, 42].

There are also anti-nutritional factors (ANFs) in plant-based beverages, which negatively affect the sensory profile as well as the bioavailability of macro- and micro- nutrients and protein quality [43]. ANFs are mainly oligosaccharides from the raffinose family, protease inhibitors, saponins and phytates. Another problem may be the presence of "beany" and "painty" off-flavors, originating from lipoxygenase activity [36]. It is possible to exclude these negative factors present in plant-based beverages as a result of various types of treatment, especially fermentation. Fermentation with LAB often results in an improved nutritional profile mainly due to the release of amino acids and bioactive compounds, decrease of ANF activities and enhancement of the protein digestibility [4, 43]. Furthermore, fermentation of milk substitutes improves sensory perception because it decreases the beany off-flavor of plant materials and provides desirable volatile flavors [1].

FERMENTATION OF PLANT-BASED BEVERAGES USING LAB

Bacteria of the genus *Lactobacillus* are among the most important bacteria used in the food industry, food microbiology and human nutrition. They have proven health-promoting properties, as well as playing a major role in food production. Until recently, the genus *Lactobacillus* included more than 250 species of bacteria, and a whole genome sequence analysis made in 2019 has shown that members of the genus are phylogenetically interwoven with other LAB genera [18]. Currently, the genus *Lactobacillus* is reduced to only 38 species around the type species, *Lactobacillus delbrueckii*, the other lactobacilli have been transferred to types with new names (however without changing the name of the species) [60]. Among the most important functions of these microorganisms are:

– imparting a specific flavor to food during the fermentation process through the release of diacetyl, hydrogen and sulfur;
– participation in the production of fermented foods (sauerkraut, pickled cucumbers, meat);
– participation in the production of fermented beverages (wine, beer) [16].

LAB are most often found on the surface of healthy or decomposed plant material, as well as in fermented products (including raw fermented meat), sewage, water, raw juices, and silage. In addition, they colonize human and animal organisms (genital organs, oral cavity, respiratory tract, and intestines). They belong to the non-pathogenic and beneficial intestinal microflora [26].

The addition of beneficial probiotic microflora to food leads to the hydrolysis of components such as proteins and lipids, which, as a result of this process, are transformed into substances that taste, smell and modify the texture of the product. This is extremely important in the subsequent perception of the product by the consumer. The introduction of probiotic strains into plant-based beverages is also an opportunity to enrich the diets of people who do not consume traditional fermented foods, such as sauerkraut or pickled cucumbers, due to their specific taste and texture [17]. Moreover, the addition of starter cultures to plant-based beverages can minimize the need for artificial stabilizers or flavorings, affecting the final nutritional and health value of the product [49].

Consumption of fermented plant-based beverages contributes to increasing the diversity of the gut microbiota, which controls most of the human body's processes. The more beneficial gut bacteria populate the intestines, among other things, the level of short-chain fatty acids (SCFAs) responsible for anti-inflammatory effects increases [49]. Probiotic bacterial cells colonizing the large intestine affect the proper functioning of the digestive system, immune system, or hormone function. Recent studies indicate that homeostasis of the gut microbiota is responsible for brain processes and works directly through the axis-microbiota-brain. Most of the serotonin is synthesized right in the gut, which is why gut dysbiosis can lead to the development of depression or other mental disorders. All these scientific reports make fermented foods and foods enriched with probiotic strains extremely necessary in the current food market [57].

The most important and key criterion for any fermented product is a sufficiently high viability of bacterial cells in the product during refrigerated storage. The number of viable bacteria in the product should be a minimum of 10^6 CFU/mL, a level that ensures that the product is able to produce a therapeutic effect on the consumer [19]. However, it is important to note that this number should be much higher, as bacterial cells are exposed to many harmful factors during the gastrointestinal passage. The high pH of gastric juice, the action of digestive enzymes and bile salts affect the loss of viability of probiotic cells and ultimately too few bacteria enter the large intestine, which will not be able to produce a health effect in the host. Ensuring sufficiently high levels of viable bacterial cells in a product is the biggest challenge for fermented food scientists [6].

THE VIABILITY OF LAB IN CEREAL – AND PSEUDOCEREAL-BASED BEVERAGES

Cereals and pseudo-cereals are a group of foods that are often fermented. This is due to their rich composition in terms of macro- and micro-nutrients, as well as their high content of fiber, the consumption of which is essential for the proper functioning of the digestive system [64]. Whole grain products contribute to reducing the risk of type 2 diabetes, cardiovascular disease, or obesity, among others [30]. Grains have been used to make traditional fermented beverages such as boza and mahewu popular in Africa, the Balkans and Turkey. The rise in popularity of such products in recent years has meant that the market for fermented foods is growing [10].

The cereal most often used in the production of fermented beverages is oats, which is mainly due to its delicate taste, acceptable to consumers. Not to forget the high content of fiber, essential amino acids and minerals such as magnesium, calcium, potassium and phosphorus [21]. Fermentation trials of oat beverages usually yield satisfactory results. Chen and colleagues fermented an oat with honey beverage using *Limosilactobacillus fermentum* PC1 bacteria and recorded the presence of probiotic bacteria at 7.96 log CFU/mL after 72 h of running the process. Storing the beverages under refrigeration (4°C) for 14 days did not significantly affect the loss of bacterial cell viability, which was finalized at 7.32 log CFU/mL. This study also showed that the fermentation of oats improves antioxidant capacity, as well as increases the levels of gallic acid and catechins [11]. The production and use of fermented beverages is often targeted at specific gastrointestinal disorders to alleviate existing ailments or to supplement a deficient diet, such as when gluten or dairy is eliminated from the diet. *Lactiplantibacillus plantarum* subsp. *plantarum* WCFS1 fermented oat drink has been undergoing testing to verify its safety and performance in people with celiac disease. The survival rate of the probiotic bacteria was estimated at 8.9 log CFU/mL, so the drink exhibits a therapeutic effect at the level of the gut and intestinal microbiota. In addition, it showed significant anti-inflammatory activity and lowered cholesterol [27].

In recent years, on the podium of the healthiest and most nutritious cereals, is millet grain. This cereal has been undergoing a real renaissance for some time, so much so that dietary fasts based on dishes made from millet groats have been created [38]. However, despite the many health benefits of consuming millet groats, such measures are not supported by the scientific literature. Nevertheless, millet is of enduring interest to researchers due to its high content of omega-3 and -6 acids, polyphenols, flavonoids and fiber. There are 8.5 grams of fiber in 100 grams of dry millet, making millet an excellent matrix for developing probiotic products [3]. The additional prebiotic properties are not only positively perceived by potential consumers, but when combined with probiotic bacteria will allow the creation of a synbiotic product. A lactobacillus-fermented millet beverage showed an unusually large increase in bacterial biomass during the fermentation process, from an initial level of 5.0 log CFU/mL to 11 log CFU/mL [40]. Fermentation of the millet beverage with an allergen-free starter produced an attractive fermented product. After a 28-day refrigerated storage period, the millet beverages contained more than 10^6 CFU/mL and were positively evaluated by lactose-intolerant individuals [61].

Among gluten-free grains, one of the older but still very popular representatives is rice. Its unquestionable advantage of using it for fermented beverages is the sweet taste that can be obtained in the final product. This is due to the breakdown of carbohydrates into sugars during the processing. Despite the great popularity of rice and its many varieties, the scientific literature does not provide many examples of its use in vegetable beverages [12]. Cichońska and colleagues attempted to develop a rice beverage with the addition of probiotic bacteria. Fermentation of the rice base with bacteria of the genus *Propionibacterium* did not yield the expected results, but the addition of starter cultures of the genus *Lactobacillus*, *Saccharomyces* and *Bifidobacterium* allowed for satisfactory results. Immediately after fermentation, high numbers of live bacterial cells were recorded in the rice beverage: *Lactobacillus* sp. 7.42–8.23 log CFU/mL, *S. thermophilus* 8.01–8.65 log CFU/mL, *B. animalis* subsp. *lactis* 8.28–8.50 log CFU/mL and *P. freudenreichii* subsp. *shermanii* 4.80–5.56 log CFU/mL. During the refrigerated storage period, these values slightly decreased [12].

More and more data appearing in scientific databases, talk about the use of buckwheat as a matrix for fermented products. These are usually yogurts and fermented beverages, which, due to the specific taste of buckwheat grain, may not be the first choice of consumers, but after learning about the health benefits of consuming this pseudo-cereal, it is worth considering introducing it into the diet. Kowalska and Ziarno [29] attempted to develop a buckwheat beverage fermented with industrial cultures, two of which contained probiotic strains. Despite the lack of consumer tests to determine the taste, aroma and texture of the product, they succeeded in obtaining a beverage with a sufficiently high number of live bacterial cells at the refrigerated storage stage, exceeding 10^6 CFU/mL [28].

Another pseudo-cereal whose popularity is growing in recent years is quinoa, which is very popular among vegans, vegetarians and people who cannot consume gluten due to celiac disease or non-celiac gluten sensitivity. It has been proven that including it in the diet in combination with other healthy eating habits can effectively prevent the development of lifestyle diseases [8]. Urquizo and colleagues fermented quinoa beverage using *L. plantarum* subsp. *plantarum* Q823, *Lacticaseibacillus casei* Q11 and *Lactococcus lactis* ARH74 strains, of which *L. plantarum* subsp. *plantarum* shows probiotic potential. The 6-hour fermentation process allowed the multiplication of the starter microflora to a level of 9.5 log CFU/mL. After a one-month storage period, the number of viable bacterial cells dropped to 9 log CFU/mL [35]. Fermentation of quinoa beverage using *L. plantarum* subsp. *plantarum* strain P31891 permitted equally satisfactory results. The number of viable bacterial cells of the aforementioned strain was remarkably stable throughout the storage process and averaged 12 log CFU/mL [8].

THE VIABILITY OF LAB IN LEGUME-BASED BEVERAGES

Legumes have received a lot of attention in recent years from scientists involved in the production of functional and fermented foods [14]. For centuries, these plants have been part of human nutrition and have played a significant role in folk medicine, exhibiting multidirectional medicinal effects [45]. The growing number of people switching to plant-based diets creates the need to enrich the market with new plant-based products, into which legumes, characterized by a rich composition of macro- and micronutrients, fit perfectly. They are characterized by a high protein content, depending on the species and cultivation method, ranging from 20 to 35% [9].

In the context of the development of plant-based beverages from legumes, soybeans are the most popular, which can be seen by following the novelties appearing on store shelves. The same is true for the production of fermented plant-based beverages, it is soybeans that are one of the better matrices for developing such products. Due to its high nitrogen and carbon content, which are necessary for the fermentation process, soy is a good carrier for probiotics [44]. Soy beverage fermented with *Lactobacillus acidophilus* CH-5 and *L. casei* KN 291 strains had very good survival of bacterial cells. After the fermentation process, 10^8 CFU/mL of the product was determined in the soy beverage, which is a very good result, indicating that the product is capable of producing a therapeutic effect [65]. The addition of *Bifidobacterium longum* to the fermenting microflora showed in the study a slight decrease in the number of live probiotic bacteria cells to 10^7 CFU/mL [9]. In soy beverages fermented with a single

B. longum strain BIM B-647, which cell count was 8.2 log CFU/mL at the beginning of the fermentation process was characterized by very rapid production of bacterial biomass. After the fermentation process, the number of probiotic bacteria cells was found to increase by 2 logarithmic orders [58].

A matrix less commonly used to develop fermented beverages is beans. In Poland, the "*Piękny Jaś Karłowy*" variety predominates, which was used in the study by Ziarno et al. [63] to develop a fermented beverage. The bean-based beverage was fermented with a multi-strain microflora that included: *Lactobacillus delbrueckii* subsp. *bulgaricus* ATCC 11842, *Lactobacillus delbrueckii* subsp. *lactis* ATCC 4797, *L. fermentum* ATCC 9338, *L. plantarum* subsp. *plantarum* DSM 9843, *Levilactobacillus brevis*, *L. acidophilus* La3, *Lacticaseibacillus paracasei* BGP1, *L. casei* 01, *Lacticaseibacillus rhamnosus* LH32 and *Lactobacillus helveticus* LH-B01. After the fermentation process, slight decreases in the number of viable bacterial cells were recorded and ranged from 7.5 to 8.7 log CFU/mL. The obtained results allowed the authors to ensure the quality of bean-based beverages, which is the most important criterion during the production of such products [63]. In another study by Ziarno and co-workers, after fermentation of beverages made from germinated seeds of "*Piękny Jaś Karłowy*" beans with two starter cultures, a fermented product meeting the criterion of minimum therapeutic level was obtained. The number of viable bacterial cells after 28 days of refrigerated storage was > 10^6 CFU/mL [62]. Fermented beverages with pea and rice proteins have an equally stable microbiotic quality. A high number of live bacterial cells was recorded not only immediately after the fermentation process, but also after more than 4 months of refrigerated storage, when the number of live probiotic cells was 8.4 log CFU/mL. This is one of the better results that have been obtained in experiments on vegetable fermented beverages [2].

Legumes with high nutritional value also include lentils, which are characterized by high amounts of soluble, well-digestible proteins [52]. Like soybeans and peas, lentils are an excellent addition to the diets of people on vegetarian and vegan diets. The main purpose of developing fermented plant-based beverages is the desire to add variety to the diets of people on plant-based diets or those with specific gastrointestinal conditions. Dietary variety translates directly into a variety of bacteria that make up the intestinal microbiota. The addition of *L. acidophilus* ATCC 4356, *L. fermentum* DSM 20052 and *L. paracasei* subsp. *paracasei* DSM 20312 strains to a lentil beverage allowed the development of a product with a live bacterial cell content of 7 log CFU/mL after 28 days of refrigerated storage. The study presented here was the first performed using lentils as a matrix [55].

THE VIABILITY OF LAB IN NUT-BASED BEVERAGES

Nuts are very popular matrices for developing plant-based beverages, which provide an excellent base for the development of beneficial probiotic microflora. Nuts subjected to the fermentation process gain not only new flavor and aroma, but also their nutritional value increases [37]. One of the most popular nut-based milk substitutes on the market is cashew beverages. Shori et al. [47] investigated the effects of three strains of probiotic *Lactobacillus* spp. such as *L. rhamnosus*, *L. casei*, or *L. plantarum* in co-cultures with *Streptococcus thermophilus* and *L. delbrueckii* subsp. *lactis* on the changes of viability of LAB in yogurt made from cashew milk. All the tested samples showed high LAB survival during the 21-day storage - in the range of 8.04–8.30 log CFU/mL for *S. thermophilus* and 6.15–6.59 for *Lactobacillus* spp [47]. Similarly high survival of bacteria during fermentation was demonstrated by Bruno et al. [7] who evaluated the cashew nut milk as a matrix to deliver commercial probiotic strains (*Bifidobacterium animalis*, *L. acidophilus* and *L. plantarum*). High survival of the tested probiotic bacteria (> 8 log CFU/mL) was reported during 30 days of storage. A significant pH decrease was observed, but it did not affect the beverage's sensory acceptance, indicating that cashew nut milk is an adequate vehicle for delivering probiotics [7].

Another raw material that has dominated the plant-based beverage market in recent years is almond. The almond-based beverage was primarily introduced and marketed as an alternative milk beverage for children and adults suffering from health conditions that include cow's milk allergy and lactose intolerance [54]. It turns out to be also a suitable matrix for the fermentation process. In an almond beverage fermented with three different LAB isolates, bacterial counts ranged between 8.97 to 9.12 log CFU/mL after 24 hours of incubation [56]. High viability of probiotic bacteria was recorded also during storage in almond beverages fermented with *Limosilactobacillus reuteri* ATCC 55730 and *S. thermophilus* CECT 986. In each of the 31 samples, the number of viable bacterial cells exceeded 7 log CFU/mL after 28 days of refrigerated storage [5].

Among the nut-based beverages that may also be a suitable fermentation matrix are peanut-based milk substitutes. Peanut products have nutritional significance as they are rich in protein, minerals, and essential fatty acids such as linoleic and oleic acids, valuable components in human nutrition [42]. In a study by Utami et al. [53] the fermentation of peanut beverage was investigated using the probiotic strain of *L. paracasei* SNP-2. The numbers of LAB showed no marked reduction in the fermented peanut milk beverages during storage at 4°C for 21 days (>8 log CFU/mL), still sufficiently high to exert beneficial probiotic effects in the host [53]. Fermentation of the peanut beverage with the use of *Bifidobacterium pseudocatenulatum* G4 also allowed obtaining a product with probiotic bacteria high survival after 14 days of storage, which was above 6.60 log CFU/mL [25].

THE VIABILITY OF LAB IN SEED-BASED BEVERAGES

Seeds are an interesting raw material for the production of milk substitutes due to the fact that they are a good source of protein, minerals, fiber and oil. They are also known for their high antioxidant activity [48]. Research into seed-based beverages fermentation is limited and focuses mainly on the flaxseed-based beverages. Łopusiewicz et al. [32] investigated flaxseed oil cake as a potential substrate for the production of a novel kefir-like fermented beverage. Commercial kefir grains containing *Lactococcus lactis* subsp. *cremoris*, *L. lactis* subsp. *lactis* biovar *diacetylactis*, *Leuconostoc mesenteroides* subsp. *cremoris*, *L. delbrueckii* subsp. *bulgaricus*, and *Saccharomyces cerevisiae* were used in the fermentation process. During 21 days of storage, the bacterial and yeast counts were maintained in the samples over the recommended for kefir level $>10^7$ CFU/mL and $>10^4$ CFU/mL for bacteria and yeast, respectively [32].

Flaxseed-based beverages fermented with the *L. rhamnosus* GG turned out to be effective in making an innovative probiotic drink. During storage at 6°C for 48 hours the viability of *L. rhamnosus* GG in all the tested samples was over the recommended probiotic minimum level ($> 10^6$ CFU/mL). Moreover, the fermentation improved antioxidant activity, polyphenolics and flavonoids content, whereas the viscosity of the samples decreased [34]. Similar results were obtained for flaxseed-based beverages fermented with *S. thermophilus*, *L. delbrueckii* subsp. *bulgaricus*, *L. acidophilus,* and *Bifidobacterium lactis* [33].

Another type of seed-based milk substitute that has been successfully fermented with LAB was hemp beverage. The hemp beverage was fermented with *L. casei* subsp. *rhamnosus* LCR 3013 and analyzed after their fermentation as well as on day 7, 14, and 21 of storage at a temperature of 4°C. On day 21 of cold storage, the number of viable *L. casei* cells in the tested beverage was at 7.35 log CFU/mL and met the therapeutic criterion. The authors concluded that the analyzed non-dairy beverages represented a category of novel food products, and their manufacture would contribute to the sustainable development of food production and the assurance of food safety [50].

THE VIABILITY OF LAB IN OTHER PLANT-BASED BEVERAGES

Among the other milk substitutes that do not fall into the above-described categories is mainly coconut beverage, which is the liquid that is extracted from the grated coconut white meat, which is rich in saturated fats, and is widely consumed in parts of Asia and South America [54]. Plant-based beverages based on matrices such as coconut are characterized by a mild taste and are often accepted by children as well [51]. Currently, the scientific literature has limited sources regarding information on fermented coconut beverages; many more reports can be found on pasteurized "coconut milk"-cow's milk alternatives. Researchers Mauro and Garcia [37] attempted to ferment coconut beverage with fairly common bacterial strains *L. reuteri* LR 92 and *L. reuteri* DSM 17938. The number of viable bacterial cells in stored samples of the plant-based beverages ranged from 6.87 to 8.50 log CFU/mL, with results below 7 log CFU/mL reported in only 2 of 24 samples [37]. Coconut water was also tested as a base for the development of beneficial starter microflora. A typically yogurt-like species of bacteria, *L. casei* L4, was added to the coconut matrix and reached a count of 9.47 log CFU/mL immediately after fermentation, while the count dropped to 7.84 log CFU/mL after a 28-day of refrigerated storage. In addition to very good bacterial survival, the starter microflora led to an increase in vitamin B12 levels [22].

CONCLUSIONS

The public's growing awareness of healthy eating, as well as the role the gut microbiota plays in maintaining health, has led to the development of such a food industry as functional foods. The biggest challenge facing manufacturers of fermented plant-based beverages is to ensure that the number of probiotic bacteria responsible for causing the health effect is high enough. The survival rate of individual bacterial strains depends on the type of plant matrix used, as well as the availability of nutrients. Not enough carbon and nitrogen is one of the reasons for insufficient growth of bacterial biomass, due to the inefficient fermentation process. The technological process itself, the fermentation temperature as well as the final pH of the product have the greatest influence on the final number of viable bacterial cells in fermented plant-based product. Scientists are working to find the best way to keep bacterial cell viability as high as possible in various types of products. The current interest in plant matrices is justified by the growing popularity of plant substitutes for animal-based food, including fermented plant products. The use of appropriate technological processes may allow to obtain products with high nutritional value and health-promoting properties, which are intensified by the addition of probiotics.

PODSUMOWANIE

Rosnąca świadomość społeczeństwa na temat zdrowego odżywiania, jak również roli jaką odgrywa mikrobiota jelitowa w utrzymaniu zdrowia, doprowadziła do rozwoju takiej gałęzi przemysłu spożywczego jaką jest żywność funkcjonalna. Największym wyzwaniem jakie stoi przed producentami fermentowanych napojów roślinnych jest zapewnienie odpowiednio wysokiej liczby bakterii probiotycznych, które są odpowiedzialne za wywoływanie efektu zdrowotnego. Przeżywalność poszczególnych szczepów bakteryjnych jest uzależniona od rodzaju użytej matrycy roślinnej, jak również dostępności składników odżywczych. Zbyt mała ilość węgla oraz azotu jest jedną z przyczyn niedostatecznego wzrostu biomasy bakteryjnej, ze względu na mało wydajny proces fermentacji. W największym stopniu na finalną liczbę żywych komórek bakteryjnych w roślinnym produkcie fermentowanym ma wpływ sam proces technologiczny, temperatura fermentacji, jak również końcowe pH produktu. Naukowcy pracują nad znalezieniem najlepszego sposobu na zachowanie maksymalnie wysokiej żywotności komórek bakteryjnych w różnego rodzaju produktach. Obecne zainteresowanie matrycami roślinnymi jest uzasadnione rosnącą popularnością roślinnych zamienników produktów odzwierzęcych, w tym roślinnych produktów fermentowanych. Zastosowanie odpowiednich procesów technologicznych może pozwolić na otrzymanie produktów o wysokiej wartości odżywczej i właściwościach prozdrowotnych, które są intensyfikowane poprzez dodatek probiotyków.

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