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Health promotional apple as an ideal substrate for probiotic beverages

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ABSTRACT

Most probiotic foods are based on dairy products. However, some people, including children, are characterized by intolerance to milk sugar- lactose. Probiotic-rich fruit juices are a good alternative to dairy products as they are free of lactose and cholesterol. Among the probiotic microorganisms, the genus of *Lactobacillus* is most often used in the production of food products. Functional food obtained in this way is also available to vegetarian consumers. The development of non-dairy probiotic products has been widely studied. The focus is made using fruits, vegetables, and grains as raw materials. This paper explores the benefits of fortification of fruit juices with probiotics using autochthonous microorganisms that not only alter the taste of drinks but also preserve the nutritional content of the fruit. It also describes the factors that affect the viability of strains of probiotic microorganisms in juices during production.

Keywords: Health, Functional foods, Apple, *Lactobacillus*, Probiotic fruit beverages, Raw materials.

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Introduction

The increase in demand for probiotic products and the urgency of problems related to lactose intolerance have led to the development of non-dairy probiotic beverages [1]. By the data suggested in 2019, about 65-70% of the world population is expected to be lactose intolerant [2]. Hippocrates first described lactose intolerance 2400 years ago, but clinical symptoms have only been recognized for the last 50 years [3]. Lactose intolerance is a deficiency of the genetically determined enzyme beta-galactosidase (lactase), which makes impossible to break down lactose into monosaccharides – glucose and galactose [4]. Symptoms such as abdominal discomfort, bloating or gas, spasm, and diarrhea are common in lactose intolerant people [5]. One way to solve this problem is to take probiotics the effectiveness of which depends on the number of lactic acid bacteria and lactose in the product. Lactose intolerance is prevalent in Europe. The percent-

age has been rising quickly to 17% in Finland and northern France. It is over 50% in South America, Africa, and Asia, and in some Asian countries, it reaches almost 100% [6]. It is clear, that lactose intolerance is very spread over the world and creation of substitute food for such consumers is important. As a result, there is increasing market need for probiotic non-dairy products. In addition, with the growth of vegetarian consumers, there is a high demand for plant-based probiotic products.

Comparing to other probiotic microorganisms, the genus of *Lactobacillus* is the most frequently used in the production of food. The development of non-dairy probiotic products has been extensively studied, including the emphasis on the use of raw fruits, vegetables, and cereals [7]. According to the Food and Agriculture Organization of the United Nations (FAO) and World Health Organization (WHO), probiotics are defined as “living microorganisms that, when present in adequate amounts, have a positive effect on the health of the host or-

ganism” [8]. The recommended concentration of probiotics is at least 10⁸ to 10⁹ colony-forming units per milliliter (CFU/ml) to maintain a minimum intestinal concentration of 10⁶ to 10⁷CFU / ml [9-11].

There are important criteria that must be met by selected probiotic strains: Non-pathogenic and non-toxic [12]; representative of the normal human microflora [13]; ability to survive in the human gastrointestinal tract, namely resistance to salivary enzymes, gastric acid pH, pancreatic juice and bile salts [13,12], adhesion, colonization and making stable population to the intestinal epithelium [4,13]; Durable enough to withstand commercial production, processing, and distribution [14]; Production of antimicrobial compounds, bacteriocins against pathogens [15]; To have a beneficial effect on the host organism [16]; It should also have good sensory characteristics to avoid unpleasant flavor and texture [17,18].

In addition, probiotics should be able to hydrolyze substances in fruits and vegetables that cannot be processed by the host, such as fructans and galactans, fructooligosaccharides (FOS), mannanoligosaccharides (MOS), xylooligosaccharides (XOS), inulin and some antinutritional factors as tannins [19,20]. They must produce useful components such as vitamins, antioxidants and antimicrobial substances such as organic acids (lactic acid and acetic acid), hydrogen peroxide, bacteriocins, aldehydes, acetoin, carbon dioxide, reuterin, reutericyclin, phenolic acids, peptides, and short chain fatty acids [21].

For example, a study by Petrov, Popova, and Petrova found that *Lactobacillus paracasei* DSM 23505 had the ability to produce large amounts of lactic acid by simultaneous saccharification and fermentation of inulin (SSF) [22]. The probiotic properties of *Lactobacillus Plantarum* MTCC 1325, a strain of *Lactobacillus*, have also been found to be associated with the reduction of Alzheimer’s disease by the production of acetylcholine [23]. Because acetylcholine is a neurotransmitter that transmits nerve signals, its decreased levels have strong connections with the progression of Alzheimer’s disease [24].

Probiotic fruit beverages as functional foods: benefits over dairy probiotics

Functional food refers to food whose nutritional level is improved in various ways. One of them is to

add probiotics [8]. For this purpose, different substrates such as cheese, powdered milk, ice cream, dairy desserts, butter, mayonnaise, and fermented vegetable foods are used [25]. However, the presence of allergens, lactose intolerance, fat content, and cold storage requirements are limitations of probiotic dairy products [26, 27]. This aspect has led to the creation of new probiotic products based on non-dairy substrates. Various raw materials [28] such as fruits, vegetables, wheat, and legumes have been studied for this purpose. Fruits and vegetables are considered to be one of the best sources as they contain minerals, fiber, vitamins and antioxidants [29]. They are also allergy free and easily available [30]. They are characterized by low cholesterol and have a vegan-friendly status [31].

According to literary data, the functional food market is one of the main attractive areas for research and innovation in the food sector. Probiotics are included in a variety of foods, such as dietary supplements, functional foods, and animal feed, as well as therapeutic and prophylactic health programs. Based on a recent market research report the market for probiotics is evaluated at USD 49.4 billion in 2018, and will reach USD 69.3 billion by 2023 [32]. Developing probiotic juices using plant substrates without interfering with the sensory side is an innovative idea for the development of functional juices. These juices are enriched with active components such as probiotics, prebiotics, phenols, flavonoids, and antioxidants [30] that are naturally present in fruits and vegetables and attract people of all ages [33]. The microbial composition of fermented juice improves gastrointestinal health and is responsible for maintaining the achieved results [34]. Given the above facts, fruits and vegetables can be used as a potential substrate for probiotic drinks [35].

The benefits of fortified fruit juices with probiotics are: [36]

- Rich in nutrients (vitamins, antioxidants, and polyphenols);
- Taste properties that attract attention for people of all ages;
- Consumed regularly, which is needful for the beneficial effects associated with probiotics;
- Considered a healthy product by the population, mainly due to its fortification with vitamins or calcium;
- Free from starter cultures that compete with probiotics in food source;

- Generally, contain antioxidant ingredients, such as ascorbic acid, to help create anaerobic conditions;
- Contain significant amounts of sugars that can be converted by the beneficial cultures;
- Do not contain allergens such as lactose and casein, which interfere with the consumption of dairy products by some population;
- Naturally free from cholesterol;
- Their movement through the gastrointestinal tract is relatively rapid, which reduces the exposure to probiotics in the host environment, such as gastric acidity.

Preparation of probiotic fruit beverages and approaches for improvement of their probiotic properties

In recent years, lactic acid bacteria have been added to fruit or vegetable juices to make probiotic drinks. Lactic acid fermentation not only changes the flavor of beverages but also preserves the nutritional composition of fruits and vegetables [37].

However, it is important to create an appropriate environment for probiotic strains in the juice to have a positive effect on human health. Many reviews on probiotics emphasize that they cannot be maintained after processing, storage, and digestion [10], which naturally affects both the quality of the product and the economic side of the manufacturer. There are several stages that can affect the viability of probiotic bacteria. In addition to being sensitive to technological processes, they must withstand the acidic environment of the stomach and the bile salts of the small intestine before reaching their destination [38]. They must also be resistant to enzymes, such as lysozyme present in the intestine, as well as to toxic compounds including phenols. They arise during digestion. Antibiotics, bacteriophages, and anaerobic conditions also affect the viability of probiotics [39]. Among the processing parameters that affect the activity of bacterial strains are pH, titratable acidity, water activity, incubation temperature, sugar content and chemicals such as hydrogen peroxide, molecular oxygen, bacteriocins, artificial flavoring, coloring agents, heat treatment, rate and proportion of inoculation, strain species, packaging materials and conditions, storage methods [40].

Numerous attempts have been made in various food products to improve their growth and viability during their production and storage [41]. Strategies include not only the strains having the desired prop-

erties selected in accordance with the above parameters but also the microencapsulation and synthesis of micronutrients such as peptides and amino acids [42].

Among the various probiotics, it is generally found that lactobacilli have the ability to resist and survive in fruit juices, with a pH of 4.3 to 3.7, while bifidobacteria are less acid-tolerant; even a pH of about 4.6 is unfavorable for their survival [43]. According to Ranadheera et al. the integration of LAB in fruit juices at low pH may increase the resistance of bacteria to stressful acid conditions [44]. Problems with adding probiotic cultures to some fruit juices can be solved by mixing with small amounts of other juices. For example, when *Lactobacillus* strains are added to fruit juices that contain citric acid in orange juice and malic acid in apple juice, bacteria convert these acids to produce CO₂, acetic acid, and lactic acid. Addition of 5% cherry juice to orange juice helps to prevent flatulence after 3 weeks and does not significantly affect cell counts of *L. plantarum* HEAL9 and *L. paracasei* 8700:2 at 8 ° C for 4 weeks [45].

As for commercial production, probiotic culture is added to the fruit juices after heat treatment because the microorganism is sensitive to high temperatures. Usually, the probiotic culture is added to the juice in an activated form, after the sequential multiplication of the lyophilized culture in the (MRS) broth. Costa et al. also suggested the use of orange juice as a substitute for MRS broth, and studies have shown that orange juice is a suitable substrate for the propagation of *Lactobacillus paracasei* [46]. It should also be noted that the addition of probiotics to juices may require special technology, as the microorganism may not be able to withstand a number of processing processes (e.g., pasteurization temperature) and raw material conditions (e.g., acidity). Hansen established a flexible formulation system in 2005 according to which probiotics are added directly to the finished liquid product. The technology uses an aseptic dosing apparatus that allows bacteria to be added only before packaging [47].

Apple as an ideal substrate for probiotic fruit beverages

Among fermented fruit juices, the production of apple juice is wide spread. Properties for apples as raw materials, different techniques of production of apple juices, and environmental conditions give the research specificity [48]. Ninety percent of fer-

mented food is produced from natural, autochthonous microflora [49]. The surface of the apple is a natural reservoir of both yeasts and bacteria [50]. In 2013, Shade, McManus and Handelsman studied the microbiome of apple flower. They described the existence of different bacterial backgrounds that develop differently from bud to fruit [51]. This study highlighted that the apple flowers are carriers of bacteria that can be used in the juice making process (mainly successfully are used Lactobacillaceae and Acetobacteraceae families) [48].

Cultivation methods play an important role in the formation of microbial composition, both in terms of abundance and diversity. The condition of organically and pesticide-poisoned apples differs radically in terms of microorganisms [52, 53]. The organic apple phyllosphere is distinguished with more abundance [54]. The microflora of the raw material is also strongly influenced by the sampling techniques, the sorting quality, and the storage conditions. The advantage of collecting apples from the ground was determined from the point of bacterial diversity [48].

Thanks to their taste and aroma, apples allow the production of probiotic juice. For this purpose, Pereira et al. [55] used Cashew apples as a substrate,

and the juice was fortified with *L. casei*. Oligosaccharides are formed during the fermentation of cashew apple juice. This fruit showed good growth for *L. casei* compared to dairy products. The fermented probiotic drink of cashew apple can be stored for 42 days in refrigerated conditions [55], which underlines its potential for commercialization.

Current market of probiotic fruit juices

There are already several new non-dairy probiotic drinks on the market. Proviva was the first probiotic product, which does not contain milk or milk components. It was launched in Sweden by Skane Dairy in 1994. The product is lactic acid-fermented oatmeal that was mixed with fruit juice. Melted grain culture was added to increase the liquid mass of the product, and *Lactobacillus plantarum* to carry out fermentation. This formula is used as the active ingredient in a food product in which 5% of oatmeal is mixed with a fruit drink with final content of *L. plantarum* 5×10^{10} CFU/L [56]. Table 1 gives some examples of non-probiotic products presented in the international market. Table gives some mixed with a fruit drink with final content of of *L. plantarum* 5×10^{10} CFU/L.

Table. Some probiotic fruit drinks, which are commercially available [38]

Probiotic Product	Probiotic Microorganisms	Company
Biola	<i>Lactobacillus rhamnosus</i> GG	Tine BA, Norway
Bioprofit	<i>Lactobacillus rhamnosus</i> GG, <i>Propionibacterium freudenreichii</i> , Shermanii JS	Valio Ltd., Finland
Bravo Friscus	<i>Lactobacillus plantarum</i> HEAL9, <i>Lactobacillus paracasei</i> 8700:2	Skanemajerier, Sweden
Gefilus	<i>Lactobacillus rhamnosus</i> GG	Valio Ltd., Finland
GoodBelly drink	<i>Lactobacillus plantarum</i> 299v	Next Foods, Colorado
Healthy life probiotics	<i>Lactobacillus paracasei</i> 8700:2, <i>Lactobacillus plantarum</i> Hea19	Golden circle, Australia
Rela	<i>Lactobacillus reuteri</i> MM53	Biogaia, Sweden

Despite the variety and popularity of Georgian juices, there are no probiotic juices on the current market in Georgia. The production of this type of juice on our market appears to be beneficial both from an economic point of view and that of public health. Besides, apple from fruit crops account

for 50% of the fruit produced by the local market in Georgia [57]. Regard to this fact, of particular interest could be apples as a substrates producing probiotic fruit beverage.

Economic and social context of Georgia, the Georgian agrifood economy and consumption pat-

terns. Georgia is still a country in transition, still under the influence of its past, i.e. the past associated with the Soviet Union. Growing domestic and external demands contributed to the economic growth, which has led the country to increase consumption, exports, and tourism. Gross Domestic Product (GDP) remained stable (5.1%) in 2019 thanks to several factors: good investment, increased government spending, good structural reforms, and the country's growing integration into regional and global economies. According to the updated forecast of the International Monetary Fund, from 14th April 2020, due to COVID19, GDP growth is expected to fall to 4% in 2020 and reach 3% in 2021, depending on the post-pandemic global economy situation [58].

According to the latest data of the International Monetary Fund, the unemployment rate in Georgia in 2019 was 11.6%. Poverty has almost halved in recent years, thanks to social policies and economic growth, but it has remained at the same level due to employment problems (16.3% in 2017, according to the World Bank data). Inequality remains high compared to other economies in the region, although a downward trend is still observed in this direction [58].

There are more than 2.7 million employed people out of 3.7 million in Georgia (according to the World Bank). The country has an agricultural tradition that has contributed to the development of the country's economy over the years. However, the agricultural sector has decreased since 1995. Today, 6.7% of GDP belongs to agriculture (World Bank). Ninety-eight percent of farmers are self-employed and production is mostly for self-consumption. More than 40% of the territory of Georgia is considered to be agricultural land, which also includes pastures and grasslands. The main agricultural products are cereals, early and late vegetables, technical or industrial crops, subtropical plants, fruit varieties, melons and pumpkins, tobacco, and grapes, rice, and tea. Stock raising is also developed and the population has livestock. Georgia is also one of the oldest regions in terms of wine production and is rich in its drinking water resources. Georgia has signed the Deep and Comprehensive Free Trade Agreement (CFTA) with the European Union, which stipulates that all Georgian agricultural products can be exported to the EU market without any commitments [58].

Since the industrial sector shrank both during the collapse of the Soviet Union and from 2004 to

2008, there has been a moderate growth trend in recent years. Thanks to it, the GDP is now 19.9%, and the number of employed people is 13.2% (World Bank). The industrial sector mainly includes food processing and the production of chemicals and textiles [58].

Currently, the goal of the Government of Georgia is to take care of the development of the country's agricultural infrastructure in terms of processing and storage of agricultural products, which will help increase the productivity and competitiveness of Georgia's agriculture. Achieving this can be possible by attracting investment in this area, and these efforts will be expected to meet local demand and increase the country's export potential [59].

An important link in achieving this goal is the various organizations, in particular the Agricultural Efficiency Recovery Project (USAID / REAP), funded by the United States Agency for International Development (USAID). It contributes to rural income growth and job creation by attracting investment in agribusiness and providing technical assistance [60]. Within the framework of the "Enterprise Georgia" project, the Georgian Beer Company introduced the production of Georgian natural fruit juices using a new, aseptic method [61]. However, there appears to be scope for more innovative food product developments and probiotic beverages could be one of them.

Conclusion

Creating a functional food using lactic acid bacteria and fruit substrates as prebiotics makes it possible for a wide range of people to get the desired benefits. Technologies are developed based on scientific research that help to avoid as much as possible the reduction of the activity of probiotic strains during food processing, which affects the quality of the final product and the economic prospects for entrepreneurs as well. The problems described in the article and the ways to solve them underline the view that these types of products have a promising future and could contribute to the development of the agrifood and economy sector of countries such as Georgia.

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