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Strains and Sugar Contents of Food Products Fortified with Probiotics

Bengi Bayer, Nuran Usta, Özlem Üstün Aytekin ✉

University of Health Sciences, Hamidiye Faculty of Health Sciences, Department of Nutrition and Dietetics, 34668, Istanbul, Türkiye

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✉ *Corresponding author (Yazışmalardan Sorumlu Yazar): ozlem.aytekin@sbu.edu.tr (Ö. Üstün Aytekin)*
+90 216 418 96 16 +90 216 346 36 40

ABSTRACT

An increasing interest in probiotic-fortified foods today can be attributed to factors such as the pandemic, climate change, and modern lifestyles. In the process of incorporating probiotics into foods, several crucial factors must be considered, including the selection of the appropriate strain, ensuring survival in the gut, production techniques, and storage conditions. The sugar content of these products is equally important, as it can significantly affect the survival and effectiveness of probiotics. Some products in the market may contain excessive amounts of sugar to make them more appealing to consumers. This study aims to determine the specific strains and sugar content in commercially available probiotic-fortified foods. Additionally, it seeks to investigate the impact of sugar on probiotics and evaluate the sugar levels in these products based on recommended dietary guidelines. Literature review indicated that strain selection and sugar content in probiotic-fortified foods could play a crucial role in the viability of probiotics and their health effects. High sugar content could negatively impact gut microbiota balance and reduce probiotic survival, emphasizing the necessity for consumers and food manufacturers to carefully assess total and added sugar levels. Further investigation into the interactions between different probiotic strains and sugar types is highly recommended for improving product formulations and ensuring the long-term health benefits of probiotics.

Keywords: Probiotic-fortified food, Probiotic, Sugar, Viability, Health effects

Probiyotiklerle Desteklenmiş Gıdalardaki Suşlar ve Şeker İçerikleri

ÖZ

Günümüzde probiyotiklerle desteklenmiş/güçlendirilmiş gıdalara olan ilginin artışı, pandemi, iklim değişikliği ve modern yaşam tarzı gibi faktörlere bağlanabilir. Probiyotiklerin gıdalara dahil edilmesi sürecinde, doğru suşun seçilmesi, bağırsakta hayatta kalma yeteneğinin sağlanması, üretim teknikleri ve saklama koşulları gibi önemli faktörler dikkate alınmalıdır. Bu ürünlerde bulunan şeker miktarı da eşit derecede önemlidir, çünkü probiyotiklerin hayatta kalmasını ve etkinliğini önemli ölçüde etkileyebilir. Piyasadaki bazı ürünler, tüketicilere daha cazip hale gelmesi için aşırı miktarda şeker içerebilmektedir. Bu çalışma, ticari olarak erişilebilir probiyotik ilaveli gıdalardaki spesifik suş çeşitlerini ve şeker içeriğini incelemeyi amaçlamaktadır. Ayrıca, şekerin probiyotikler üzerindeki etkisini araştırarak bu ürünlerdeki şeker seviyelerini önerilen kılavuzlara göre değerlendirmeyi hedeflemektedir. Literatür derlemesi, probiyotiklerle desteklenmiş gıdalarda suş seçimi ve şeker içeriğinin, probiyotiklerin canlılığı ve sağlık üzerindeki etkileri açısından önemli bir rol oynadığını ortaya koymaktadır. Yüksek şeker içeriği, bağırsak mikrobiyota dengesini olumsuz yönde etkileyebilir ve probiyotiklerin hayatta kalmasını azaltabilir, bu da tüketiciler ve gıda üreticileri için toplam ve ilave şeker seviyelerinin dikkatle değerlendirilmesi gerekliliğini vurgulamaktadır. Farklı probiyotik suşları ve şeker türleri arasındaki etkileşimin daha ayrıntılı incelenmesi, ürün formülasyonlarının geliştirilmesi ve probiyotiklerin uzun vadeli sağlık faydalarının sağlanması açısından önerilmektedir.

Anahtar Kelimeler: Probiyotikli gıda, Probiyotik, Şeker, Canlılık, Sağlık etkileri

INTRODUCTION

The ecosystem of microorganisms living in a particular environment is called the "microbiota". Similar to the human microbiota, the soil microbiota is the living ecosystem of the earth and consists of a wide variety of organisms. Environmental pollutants introduced in modern life have a negative impact on soil microbiota. A decline in the nutritional value of foods is a result of these negative alterations in the soil. Macro and micronutrient deficiencies are gradually increasing in modern people who are fed low-nutritional-value foods [1]. In addition, industrial toxins, a western-style diet, and a sedentary lifestyle cause dysbiosis in which the balance of the intestinal microbiota is disturbed [2]. In the last century, the need for foods that provide benefits beyond nutritional function has increased to modulate nutritional deficiencies and impair gut microbiota. In addition, consumer demand for high-quality meals and awareness of healthy living have led to the development of functional foods. Functional foods can be created by changing the content of food using different engineering and biotechnological methods or by adding bioactive components, such as phenolic compounds, vitamins, minerals, and fiber [3]. Probiotics, which are beneficial bacteria, have also been used for this purpose. Probiotics are known to strengthen the immune system and improve overall health by regulating human gut microbiota [4]. Food formulations containing probiotic bacteria have an important place among functional foods due to the numerous proven health benefits they provide in addition to their traditional nutritional functions [5].

Owing to functional food technology, foods that are not contain probiotic bacteria (such as tea, coffee, chocolate, and granola) are used as probiotic-fortified products. However, the selected probiotic must be well defined at the species and strain levels and be safe for consumption. Additionally, probiotic strains must be compatible with the food matrix, resistant to production processes, and maintain their viability under storage conditions. Active strains must survive until the end of the product's shelf life to provide the expected benefits from probiotics. In addition, they must survive in the low pH, bile salt, and enzymatic environment of the gastrointestinal tract to reach and colonize the intestine [6]. Therefore, not all probiotics are suitable for use in food. When choosing which probiotics to use in food formulations, the manufacturer should consider many factors [7]. The most common microorganisms found in probiotic foods are *Lactobacillus* and *Bifidobacterium* spp. However, because of their tolerance to harsh environments, spore-forming bacteria, such as *Bacillus coagulans* and *Bacillus subtilis*, are becoming more common in the food industry [8].

Sugar is added to food products to ensure the consumer acceptability of food and to improve its sensory properties [9]. Sugar is added to make nutrient-dense foods, including probiotic foods, appropriate for general consumption (Figure 1).

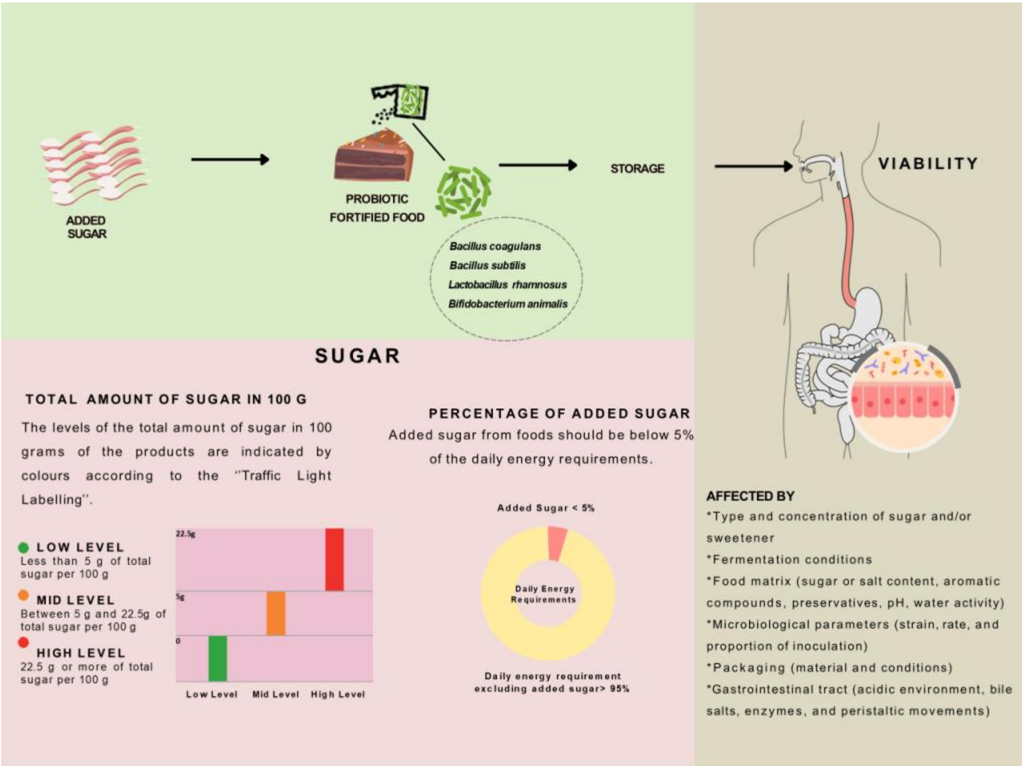

Figure 1. The effect of sugar amount on viability in probiotic-fortified foods

Health authorities agree that people can consume some sugar within their daily recommendations [10]. However, it should be noted that the nutritional composition of probiotic foods and the added active strains interact [3]. Considering the addition of different types and amounts of sugar sources to foods, the impact of the consumed sugar quantity on probiotic activity is often overlooked. The sugar content in probiotic food products should be evaluated to ensure their effective use in a healthy diet. In our study, the compositions of probiotic-containing food samples from almost every sector available on the market were analyzed and assessed in terms of their sugar content (Table 1 and Table 3). Given that studies have shown a decline in the survival rates of probiotic bacteria in dairy products produced using different sugar sources or amounts [11], the potential risks of sugar's impact on the viability of probiotic bacteria in food are a significant public health concern.

PROBIOTIC-FORTIFIED FOODS

Viability of Strains

Viability is a basic requirement for the mechanism of action and functionality of probiotics. The viability of probiotic microorganisms can be divided into two parts: food and the human body [3]. Microorganisms must survive primarily under food production and storage conditions [7]. Processing parameters, such as the strain selected during food production, fermentation conditions, food matrix (sugar or salt content, aromatic compounds, preservatives, pH, water activity), heat treatment, incubation temperature, and microbiological parameters (strain of probiotics employed, rate, and proportion of inoculation) are among the factors affecting probiotic viability. The packaging material used, and packaging conditions should also be considered as they affect the conditions of microorganisms during storage [6].

The next step is to survive the passage through the gastrointestinal tract. The acidic environment, bile salts, enzymes, and peristaltic movements in the intestinal tract are the major challenges for microorganisms [12]. Different encapsulation methods are used to protect microorganisms from harsh conditions in the gastrointestinal tract. However, there are a few important factors: the capsules used should not affect the sensory properties of the food; they should provide cell release and be protective in the gastrointestinal tract. Thus, probiotics can reach the intestine and show the expected effects [13].

The type and concentration of sugar and/or sweetener added to the product during food processing can affect probiotic viability. In a study, the impact of varying sugar and inulin levels on the viability of probiotic bacteria was investigated. They utilized different formulations of probiotic ice cream containing freeze-dried mixed cultures of *Streptococcus salivarius* spp. *thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus acidophilus* La-14, and *Bifidobacterium lactis* BL-01. This study focused on ice cream samples prepared with three different sugar concentrations (15%, 18%, and 21%) and two levels of inulin (1% and 2%). Over a 90-day storage period, the viability of probiotic bacteria in these formulations was evaluated. The results indicated that the viability of yoghurt and probiotic bacteria was influenced by inulin and sugar levels. Inulin, which serves as a food source for probiotic microorganisms, increases bacterial viability. Ice cream samples with 18% sugar concentration exhibited the highest number of viable bacteria. Conversely, the viability of the bacteria was lower at 15% and 21% sugar concentrations. Furthermore, this study emphasized that different bacterial strains react differently to the same sugar and inulin levels. *S. thermophilus* demonstrated the highest viability during storage, whereas *L. acidophilus* and *B. lactis* showed a rapid decrease in viability. In fact, at the end of the 90-day storage period, the viable numbers of *L. acidophilus* and *B. lactis* (5 log CFU/g) fell below acceptable levels (≥6 log CFU/g) [13].

Another study investigated the effects of low-calorie sweeteners as substitutes for sugar on the bacterial vitality in ice cream. Kalicka et al. [14], examined the viability of *Bifidobacterium animalis* subsp. *lactis* BB-12 strain before and after storage in nine different ice cream formulations containing various amounts of polyols (maltitol, erythritol, xylitol) and sugar. The control group was treated with an ice cream containing sucrose (15%). After 28 days of storage, all samples had a live cell count above 8 log CFU/g. The lowest vitality was observed in the sample containing 7.5% sucrose and 8.38% erythritol, whereas the highest vitality was consistently maintained in the control group. A sugar ratio of 15% yielded good vitality. This study supports the use of polyols in appropriate doses as a substitute for sugar.

To maximize the benefits of probiotic-fortified foods, pH, NaCl, sugar type/concentration, fat concentration, and storage conditions should be considered. When discussing the relationship between sugar and viability, it is important to consider that higher sugar concentrations can negatively impact both bacterial viability and sensory characteristics. In addition, the selection of specific probiotic bacterial strains plays a vital role in determining their response to these factors. Therefore, when determining the sugar levels in probiotic-fortified product formulations, it is necessary to consider the specific viability requirements of the chosen bacterial strains to ensure optimal survival. This is crucial for preserving the desired probiotic benefits in the final product and providing functional food to consumers. The number of bacteria (>7 log CFU/g) in probiotic-fortified foods must be maintained throughout their shelf life. Hence, the production process of probiotic foods should be designed to preserve the viability of probiotic microorganisms and maintain the specified bacterial count until the end of the shelf life [15].

Strain Content of Probiotic-fortified Foods

The dynamics and stabilization of the human gut microbiome during the first year of life are influenced by the mother's microbiota, diet, lifestyle, and genetics [16]. The diversity of the microbiota, which is affected by diet, lifestyle, ethnicity, geography, and environmental factors during the lifetime. Packaged probiotic- fortified products and their strain content in the current food market are listed in Table 1 as model products.

Table 1. Probiotic- fortified products and strain contents (Products in the table were found by searching the word "probiotic food" in the online database)

Probiotic Product	Brand Name	Active Strain Content
Grain And Grain Products		
Bread	The Grain Seed'licious	*B. coagulans*
Cracker	Kuna Pops	*B. coagulans*
Chickpea Granola- Wholesome Raspberry	Effi Foods	*B. coagulans*
Probiotic Oatmeal & Banana Baby Cereal	Gerber	*B. lactis*
Special K® Probiotics Berries & Peaches Cereal	Kellogs	*B. lactis*
Muesli	Sunsol	*B. coagulans*
Bar	Fropie	*B. coagulans*
Oat Bar	Nice & Natural	*B. coagulans*
Gluten Free Pancake Waffle Mix	Enjoy Life	*B. coagulans*
Baking mix Gluten Free, Nut Free, Vegan, Pizza Crust Mix	Enjoy Life	*B. coagulans*
Dairy Product		
Milk	Moringa Caldus	*Bifidobacterium longum* ssp. *longum* BB536
Lowfat Yoghurt	Nancy's	*Bifidobacterium lactis* BB-12, *L. acidophilus* LA-5, *L. rhamnosus* LB-3, *L. casei* (2 isolated strains), *L. rhamnosus* (2 isolated strains), *L. acidophilus* (2 isolated strains), *B. lactis* (2 isolated strains)
Yoghurt	Activia	*B. lactis*
Dairy Free Yoghurt	Califia Farms	*Bifidobacterium* BB-12, *S. thermophilus, L. bulgaricus*
Drinkable Yoghurt	Danactive	*Lactobacillus casei* CNCM I-1518
Kefir	Nancy's	*B. lactis BB-12, L. acidophilus LA-5, L. rhamnosus LGG, L. rhamnosus LB-3, L. casei (*2 isolated strains), *L. rhamnosus (*2 isolated strains), *L. acidophilus* (2 isolated strains), *B. lactis* (2 isolated strains)
Probiotic Drink	Yakult	*L. casei Shirota*
Probiotic Shot	Activia	*L. bulgaricus, L. lactis, S. thermophilus*
Sour Cream	Good Culture	*L. acidophilus, B. lactis*
Cottage Cheese	Sek	*L. acidophilus, B. lactis*
Products Containing Fruit and Vegetables		
Organic Vegetable and Fruit Mix	Garden of Life	*L. gasseri, L. plantarum, L. casei, L. acidophilus*
Non-Dairy Smoothie	Love Grace	*Bacillus coagulans*
Fruit Gel	Welch's	*B. subtilis*
Juice	Biola	*L. rhamnosus*
Juice	Tropicana	*B. lactis*
Juice	Valio Gafilus	*L. rhamnosus* GG
Fruit Drink	Danone Proviva	*L. plantarum*
Sorghum Cauliflower Puffs	Vegan Rob's	*Bacillus coagulans*
Fruit kombucha	Humm	*Bacillus coagulans, B. subtilis*
Soup	Presserys	*Bacillus coagulans*
Antioxidant Berry mix	Nature's garden	*L. rhamnosus* GG
Sugar Product		
Chocolate	Healthy Delights Naturals	*B. coagulans*
Lollipop	Dr. John's	*B. subtilis*
Chocolate Shake	Lyfe Fuel	*Lactobacillus acidophilus* DDS, *L. plantarum, L. rhamnosus, B. breve, B. longum*
Sugar	Sugar 2.0	*B. coagulans*
Herbal tea sweetened with molasses	Vita Biosa 10+	*B. animalis, B. lactis, L. acidophilus, L. casei, L. rhamnosus, Lactococcus lactis* ssp., *Lactis biovar. Diacetylactis, L. pseudo mesenteroides, S. thermophilus*
Drinks		
Green tea	Doğadan	*B. coagulans*
Black tea	Doğadan	*B. coagulans*
Coffee	Gut Power	*B. coagulans*
Water	Karma	*B. coagulans*
Hot chocolate	Cocosutra	*B. coagulans*
Dairy free drinkable yogurt	Califia farms	*Bifidobacterium lactis* BB-12, *S. thermophilus, L. bulgaricus*
Choco cinnamon banana vegan protein powder	Vega	*B. coagulans*
Pear digestion shot	Goodbelly	*L. plantarum* 299V
Mojita lime mint coconut sparkling drink	Kevita	*B. coagulans*
Metabolism tea	Super organics	*B. coagulans*
Turmeric ginger shot	Welo	*B. subtilis*

Table 1 presents a compilation of commercially available probiotic-fortified products categorized into grain-based items, dairy and non-dairy products, fruit and vegetable-containing foods, sugar products, and beverages. The active probiotic strains identified in these products include various species of *Bacillus*, *Lactobacillus*, and *Bifidobacterium*, which are known for their potential health benefits, particularly in gut microbiota modulation. The products in Table 1 were selected as examples from almost every category in the food and beverage industry. When the strain content of the products was examined, it was observed that *Bacillus coagulans,* *Bacillus subtilis, Bifidobacterium lactis,* and *Lactobacillus rhamnosus* strains were frequently used in the food market.

Characteristic of Strains

Bacillus coagulans, Bacillus subtilis, Bifidobacterium lactis, and *Lactobacillus rhamnosus* are the probiotic microorganisms most frequently employed in probiotic-fortified products, as demonstrated in Table 1, and they accepted GRAS status. The origins of these probiotic microorganisms, their bacterial attributes, health impacts, preferred food sources, and the benefits of their utilization are comprehensively outlined in Table 2. The strains, such as *Bacillus coagulans*, *Bacillus subtilis*, *Bifidobacterium lactis*, and *Lactobacillus rhamnosus*, demonstrate unique attributes such as resistance to stomach acid and bile, spore formation, and lactic acid production, which contribute to their viability in various food matrices. These probiotics are associated with significant health benefits, including gastrointestinal health improvement, immunomodulation, and prevention of infections, making them valuable components in both dairy and non-dairy probiotic products. Although probiotic strains generally promote gut health, specific species also provide specific benefits [17]. However, when it comes to a probiotic that provides benefits to people in food and beverages, the characteristics of probiotic bacteria might be variable [7].

Sugar in Probiotic- Fortified Products

According to WHO, the sugar naturally present in fruits and vegetables is named ‘intrinsic sugars’ and "free sugar" includes all monosaccharides and disaccharides added to foods and sugars naturally found in honey, molasses, and fruit juices [52]. Intrinsic sugars take longer to enter the bloodstream and have less of an impact on blood sugar than added sugar, owing to beneficial components such as fiber and various phytochemicals found in fruits and vegetables. In addition, while sugar increases the energy content of the diet, it also decreases the quality of the diet as it decreases the nutrient density [9].

Sugar is an essential taste component of packaged products. In a consumer report from FONA, a specific subsidiary, it was found that 70% of consumers prioritize taste over high sugar content in a product. Despite this, 50% of consumers aim to cut down on their sugar intake. As a result, the market for new products with reduced sugar has surged by 54% since 2017 [53].

Additional sugar consumption is influenced by factors such as age, sex, ethnicity, and income. Men, young adults, low-income individuals, and adolescents consume more sugar than the average population. However, in recent years, the importance of specifying the sugar levels and sources in packaged foods has increased considerably. According to the results of the survey studies, consumers want to know how much sugar is in the product and what its source is, and taste is still the most important parameter [54]. This indicates that consumers’ choices are flavor-based rather than health based. Consumer preferences for probiotic food products are also similar. Probiotic- fortified products containing added sugars have been preferred over sugar-free products. This affects the ingredients of packaged probiotic products containing added sugar [9]. However, excessive sugar (more than 5% of total daily calories from added sugar, according to WHO) consumption leads to many non-communicable diseases such as obesity, diabetes, and heart disease [10]. The negative effects of sugar on health and its increasing prevalence have resulted in organizations such as the WHO and NHS (National Health Service) making consumption recommendations on sugar and added sugar.

In this case, the content and nutritional values of packaged foods (such as calories, total carbohydrates, total fat, protein, salt, and sugar) should be known to consumers. Consumers choose food according to the nutrition labels written on the package. Therefore, nutrition labels are of great importance in influencing consumer behavior. Anastasiou, Miller, and Dickinson [55] published a systematic review of the relationship between food label use and dietary intake in adults. The presence of health claims and use of nutrition labels have been reported to result in healthier decisions. A new FDA regulation requires added sugars to be listed on the ‘nutrition facts label’ so that you can make decisions based on your unique needs and preferences. There is information such as added sugar and fiber under the term carbohydrates on the label. The term “total carbohydrates” on the labels refers to the total amount of dietary fiber, sugars that are naturally found in the foods they contain and added sugars. When reviewing nutrition labels, the primary focus should be on how many carbohydrates come from added sugar [56].

According to the traffic light labelling, a product’s total sugar content of 100 g should be less than 5 g for a low level, between 5 and 22.5 g for a medium level, and 22.5 g or more for a high level. Products with low, medium, and high total sugar are colored green, amber, and red, respectively [57].

According to the WHO [58], "Sugar Intake for Adults and Children" guidelines, added sugar from foods should be below 5% of the daily energy requirements. In Table 3, the percentage of added sugar is presented according to the percentage of daily value (DV) in the label information. Daily values are the reference amounts of nutrients to be consumed or not exceeded each day. From this point of view, as seen in Table 3, the sugar contents of probiotic- fortified products available on the market were given according to WHO guideline and traffic light labelling.

Table 2. Characteristics of strains used in packaged probiotic- fortified products

Probiotics	Origin	Properties	Health Effects	Food Products	Advantages	Ref
Bacillus coagulans	Plant-based sources	Facultative anaerobe, Gram positive, Spore forming, Lactic acid production, Growth temperature: 35-50°C (live up to 65°C), Growth pH:5.5-6.5, (grow at pH 4.5), GRAS Level	Gastrointestinal disorders (such as IBS, constipation), Preventing the high serum lipid profile, increase nutrient absorption, promoting lactose digestibility (with enzyme production), improving intestinal function in the elderly	Grain-based products (such as pasta), heat-treated vegan probiotic food products, yogurt, Fermented beverage	Heat resistant spore forms, resistant to low pH and bile, no effects on sensorial properties	[8, 18-23]
B. subtilis	GI tract (ruminant and human), Soil	Facultative anaerob, Gram positive, Spore forming, biofilm forming, enzyme production (such as amylase, protease), Growth temperature:10-55°C, (Optimum growth temperature: 37°C), Growth pH: 2.5-9, GRAS Level	Childhood diarrhea, Improving IB symptoms, Antimicrobial activity in GI and other infections, Regulation of metabolic profile, Antioxidant activity	Natto (japanese food), A wide variety of foods and beverages in addition to dairy products	Spore forming, Durable biofilm layers, No refrigeration needed for vitality, Resistant to low pH,	[24-34]
Bifidobacterium lactis	Human and mammalian large intestine	Anaerob,Gram positive, Does not form spores, Lactic acid production, Proteolytic activity, Growth temperature:25-45°C, Growth pH: 4-7, GRAS Level	Antibiotic-associated diarrhea, Immunomodulation, Preventing respiratory infection, Improving gut functions, Improving atopic eczema in infant	Probiotic dairy products, Baby foods, Dietary supplements Fermented milk products	Tolerance to high oxygen level, Resistance to stomach acid and bile salt	[35-44]
Lactobacillus rhamnosus	A wide range of habitats (dairy products, fermented meat, fish, vegetables and cereals, sewage, human gastrointestinal tract, vagina)	Homofermentative facultative anaerobe, Gram positive, Does not form spores, Production of lactic acid and bacteriocin, Growth temperature: 6-41°C, Growth pH: 4.5-6.5, GRAS Level	Preventing urinary tract infection, Increasing insulin sensitivity, Diarrhea (AAD and gastroenteritis), preventing certain allergic symptoms	Yogurt, cheese, milk, kefir and non-dairy probiotic products such as juice	Resistance to stomach acid and bile, Adhesion to mucosal cells, Extending shelf life, Ripening of cheese	[45-51]

Table 3. Packaged probiotic-fortified products and sugar contents (Prepared using the product information of the manufacturers.)

Probiotic Product	Product	Serving Size /Calorie	Sugar Amount (T.S / A.S)	Added Sugar Amount (WHO 5% Criteria)	Total Sugar/100 g	Total Sugar Level (Traffic Light Labelling
Chickpea Granola- Wholesome Raspberry	Effi Foods	30g / 140 kcal	4g / 3g	7%	13.3g	Med Level
Probiotic Oatmeal & Banana Baby Cereal	Gerber	15g / 60 kcal	2g / <1g	<1%	13.3g	Med Level
Special K® Probiotics Berries & Peaches Cereal	Kellog's	42g / 160 kcal	13g / 12g	24%	30.9g	High Level
Muesli	Sunsol	50g /196 kcal	7.7g / U	U	15.4g	Med Level
Cashew & Chia Probiotic Bar	Fropie	35g /138 kcal	13.7g / 0g	0%	39.1g	High Level
Oat Bar	Nice & Natural	35g /153 kcal	6g / U	U	17.1g	Med Level
Gluten Free Pancake Waffle Mix	Enjoy Life	40g /140 kcal	5g / U	U	12.5g	Med Level
Lowfat Yogurt Blueberry	Nanc'y	150g /120 kcal	14g / 7g	14%	9.3g	Med Level
Yogurt (plain)	Activia	100g /100 kcal	11g / 0g	0%	11g	Med Level
Trail Mix, Probiotic Fruit & Yogurt	Archer Farms	35g /160 kcal	21g / 19g	38%	60g	High Level
Danactive Drinkable Yogurt (vanilla)	Danone	93mL /80 kcal	13g / 10g	20%	14g	Med Level
Kefir (plain)	Nancy's	240mL /180 kcal	8g / 0g	0%	3.3g	Low Level
Probiotic Drink	Yakult	80mL /50 kcal	10g / 9g	18%	12.5g	Med Level
Probiotic Daily Shot (plain)	Activia	80mL /27.2 kcal	3.7g / 0g	0%	4.6g	Low Level
Peach & Tea Probiotic Daily Shot	Activia	80mL / 45 kcal	6.9g / U	U	8.6g	Med Level

*(T.S: Total Sugar, A.S: Added Sugar, U: Unknown)

Table 3 lists probiotic-fortified products categorized by low, medium, and high sugar levels. It does not provide definitive information on how these sugar levels affect the benefits of probiotics, but high sugar consumption is known to reduce intestinal bacterial diversity, potentially leading to microbiota dysbiosis. Consequently, selecting probiotic products with low or medium sugar levels, as indicated in Table 3, may support a healthier diet. Furthermore, the traffic-light labeling for sugar levels is determined based on total sugar content, making it essential to consider the amount of added sugar in each product. For instance, Table 3 shows that a low-fat blueberry yogurt contains 9.3 grams of total sugar, placing it in the medium sugar category, yet 14% of this sugar comes from added sources, which should be taken into account when making dietary choices. Another example is a drinkable yoghurt, which contains a total of 14 g of sugar and is considered a product with medium sugar levels; however, 20% of this sugar is added sugar. Both probiotic yoghurts exceeded the WHO's sugar recommendations. This situation can be a risk factor for a decrease in probiotic bacteria and increase in pathogenic bacteria. Therefore, it is important to consider both the total sugar content and amount of added sugar in probiotic- fortified products. At this point, the effect of sugar on the viability of probiotics in the products and how this effect reflects the beneficial effects of probiotics has become significant.

The impact of added sugar on the viability of probiotics in functional foods varies depending on factors such as the type and concentration of sugar, the specific probiotic strains used, and the processing conditions of the food product. As a result, research studies have been conducted using different sugar levels, various strains, and diverse food matrices to investigate the relationship between sugar and the viability of probiotics. Table 4 presents the selected research studies concerning the correlation between sugar and viability in probiotic- fortified products.

As shown in Table 4, ice cream is frequently utilized in probiotic studies due to its ability to support probiotic viability. In such products, high sugar concentrations can induce osmotic stress, potentially leading to cell damage or death. Conversely, sugar may also exert a cryoprotective effect, mitigating freezing-induced damage [59].

Shahsavan et al. [61] tested this issue using ice cream formulations with different quantities of sugar (14%, 16%, and 18%) and fat (5%, 7.5%, and 10%). The study found that the lowest number of *L.casei* cells was observed in the sample with 14% sugar and 10% fat, while the sample with 16% sugar and 5% fat had the highest number of cells. As the amounts of sugar and fat increased, the viability of *L. casei* was negatively affected. Similarly, Akın et al. [13] also examined ice cream formulations with varying sugar concentrations (15%, 18%, and 21% w/w) and observed that a relatively high sugar content could have a negative impact on probiotic viability due to excessive osmotic pressure. The best viability was observed in the sample with 18% sugar. Both studies indicate that optimal *L. casei* viability is achieved at moderate sugar concentrations.

Table 4. Studies on strain viability in probiotic- fortified products

Formulation	Probiotic Bacteria	Sugar/Amount	Probiotic Strain (log cfu/g)	Viability (log cfu/g)	Storage Conditions	Ref.
Ice cream Fresh milk =45%; cream= 15%; skimmed milk powder= 7.5%; sucrose=15%; aroma =0.1%; water =10%	*Bifidobacterium animalis subsp. lactis* BB-12	Sucrose =15%	8.38	8.31	28 days at -22 °C	[60]
Ice cream Fresh milk =45%; cream= 15%; skimmed milk powder= 7.5%; erythritol=8.38%; sucrose=7.5%; aroma =0.1%; water =10%		Erythritol=8.38% Sucrose=7.5%	8.13	8.03		
Ice cream Skim milk, cream, skim milk powder, vanilin, stabilizer and emulsifier, sugar =14%	*Lactobacillus casei*	Sucrose =14% (fat=10%)	8.80	6.23	90 days at -24ºC	[61]
Ice cream Skim milk, cream, skim milk powder, vanilin, stabilizer and emulsifier,sugar =16%		Sucrose =16% (fat=5%)	8.95	7.38		
Ice cream Whole milk=45%; fat=15%; skim milk powder=7.4%; stabilizer=0.5% vanilin=0.1%; Sugar=18%; inulin=2%	*Streptococcus salivarius* spp. *thermophilus, Lactobacillus delbrueckii* ssp. *bulgaricus Lactobacillus acidophilus* LA-14, *Bifidobacterium lactis* BL-01	Sugar=18% (inulin=2%)	*S. thermophilus*: 8.59 *L. delbrueckii* ssp. *bulgaricus*:7.79 *L. acidophilus* LA-14: 8.08 *B. lactis* BL-01: 8.14	*S. thermophilus*: < 8 *L. delbrueckii* ssp. *bulgaricus*: <7 *L. acidophilus* LA-14: <7 *B. lactis* BL-01: < 7	90 days at -18ºC	[11]
Ice cream Whole milk=45%; fat=15%; skim milk powder=7.4%; stabilizer=0.5% vanilin=0.1%; Sugar=21%; inulin=0%		Sugar=21% (without inulin)	*S. thermophilus*: 7.99 *L. delbrueckii* ssp. *bulgaricus*:7.52 *L. acidophilus* LA-14: 7.64 *B. lactis* BL-01: 7.73	*S. thermophilus*:< 8 *L. delbrueckii* ssp. *bulgaricus*: 7 *L. acidophilus* LA-14: <5 *B. lactis* BL-01: < 5		
Chocolate Cocoa butter=30%; cocoa mass;whole milk=13.95%; skim milk powder=7.80%; sugar =38.75% soy lecithin =0.30%, polyglycerol polyricinolate (PGPR)=0.20%	*L.acidophilus* LA-14	Sugar=38.75%	8.92		90 days at 20 °C	[62]
Chocolate Cocoa butter=30%; cocoa mass;whole milk=13.95%; skim milk powder=7.80%;maltitol =38.75% soy lecithin =0.30%, polyglycerol polyricinolate (PGPR)=0.20%			8.16			
Cocoa juice Water, cacao pulp =34%, sucrose=9.7%	*Lactobacillus casei* NRRL B-442	Sucrose=9.7%	8.76		42 days at 4 °C	[63]
Cocoa juice Water, cacao pulp = 34%, sucralose=0.48%		Sucralose=0.48%				
Chokeberry juice Sugar= 28.8 g/L; chokeberry juice = 100 ml;L. paracaseiSP5 =1 g freeze-dried bacterial biomass	*L. paracasei* SP5	Sugar=28.8 g/L	7.4		28 days at 4 °C	[64]
Chokeberry juice Sugar= 40.3 g/L; chokeberry juice = 100 ml;L. paracaseiSP5 =1 g freeze-dried bacterial biomass		Sugar=40.3 g/L	9.5			
Ice cream Fat= 12% ; milk solids nonfat= 11%; stabilizer emulsifier=0.32%; sugar=12.5%; corn syrup solids=4.5%	*Lactobacillus acidophilus*	Sugar=12.5% Corn syrup=4.5%	8		17 weeks at -29ºC	[65]
Ice cream Fat= 12% ; milk solids nonfat= 11%; stabilizer emulsifier=0.32%; sugar=12.5%; corn syrup solids=4.5%	*Bifidobacterium bifidum*	Sugar=12.5% Corn syrup=4.5%				

In addition to sugar, the impact of low-calorie sweeteners on probiotic activity is also a subject of interest. Therefore, sweeteners are commonly used in studies that monitor probiotic viability in food products. A study conducted on white chocolate investigated the viability of *Lactobacillus acidophilus* and *Lactobacillus paracasei* in sugar-containing and sugar-free (containing maltitol) chocolates. The study found that *L. acidophilus*-maintained cell counts above 7 log CFU/g during a 90-day storage period. *L. paracasei* initially exhibited a faster decline but stabilized at around 6.61-6.89 log CFU/g. Throughout the study, although sucrose provided better results, the cell counts in white chocolate containing maltitol was still reported to be within the therapeutic range (>6 log CFU/g) [62]. Similarly, Kalicka et al. [60] investigated the effects of sweetener and sugar on viable cell count in ice cream containing *Bifidobacterium animalis* subsp. BB-12. In the study, the highest number of viable cells was found in the control sample containing 15% sucrose, while the lowest numbers were found in the sample containing a combination of erythritol and sucrose. At the end of the 28-day storage period, it was reported that the cell counts of all ice cream samples indicated promising results, with a consistent presence of 8 log CFU/g. Gündoğdu et al [66] reported 7.49 log CFU/g for the same contidions for *Bifidobacterium animalis* subsp. BB-12.

In summary, the varying sugar content of the products determines the viability of probiotic bacteria and the sensory properties of the product. However, higher sugar concentrations do not always result in a lower count of viable bacteria. It is the responsibility of food manufacturers to find the right concentrations for both the sensory and physical properties of the product, as well as its probiotic activity. In fact, the inclusion of higher sugar amounts in probiotic-fortified products intended for health benefits is not deemed appropriate when evaluated in the context of a healthy diet [67].

In contrast, a notable portion of individuals face challenges in embracing sugar-free variants of probiotic-fortified products. For such individuals, an advisable approach might involve selecting the products featuring sugar content below 5% during the initial phase of acclimatization. The integration of these specific probiotic items, known to enhance overall health, into their dietary regimen could be deemed an advantageous strategy.

CONCLUSION

In this review, we evaluated probiotic-fortified foods across different categories available in the market based on their strain composition and sugar content. The sugar levels found in these foods often surpass the recommended daily limits for added sugar and total sugar intake as advised by the World Health Organization (WHO) and the National Health Service (NHS). According to the guidelines, opting for low-sugar options can increase both consumption and the intake of probiotics. However, the precise threshold at which excessive sugar consumption leads to dysbiosis and inhibits probiotic activity remains uncertain.

The viability of a particular probiotic strain can vary depending on factors such as the type of sugar, sugar concentration, strain type, product formulation, and food processing conditions. Conducting research that focuses on specific strains would be invaluable in understanding how different amounts of sugar impact viability – either supporting or inhibiting it. Such strain-specific investigations will shed light on the intricate interplay between sugar content and probiotic viability, offering critical insights for optimizing these foods' benefits.

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