

Perspective

# Ready to Use Therapeutical Beverages: Focus on Functional Beverages Containing Probiotics, Prebiotics and Synbiotics

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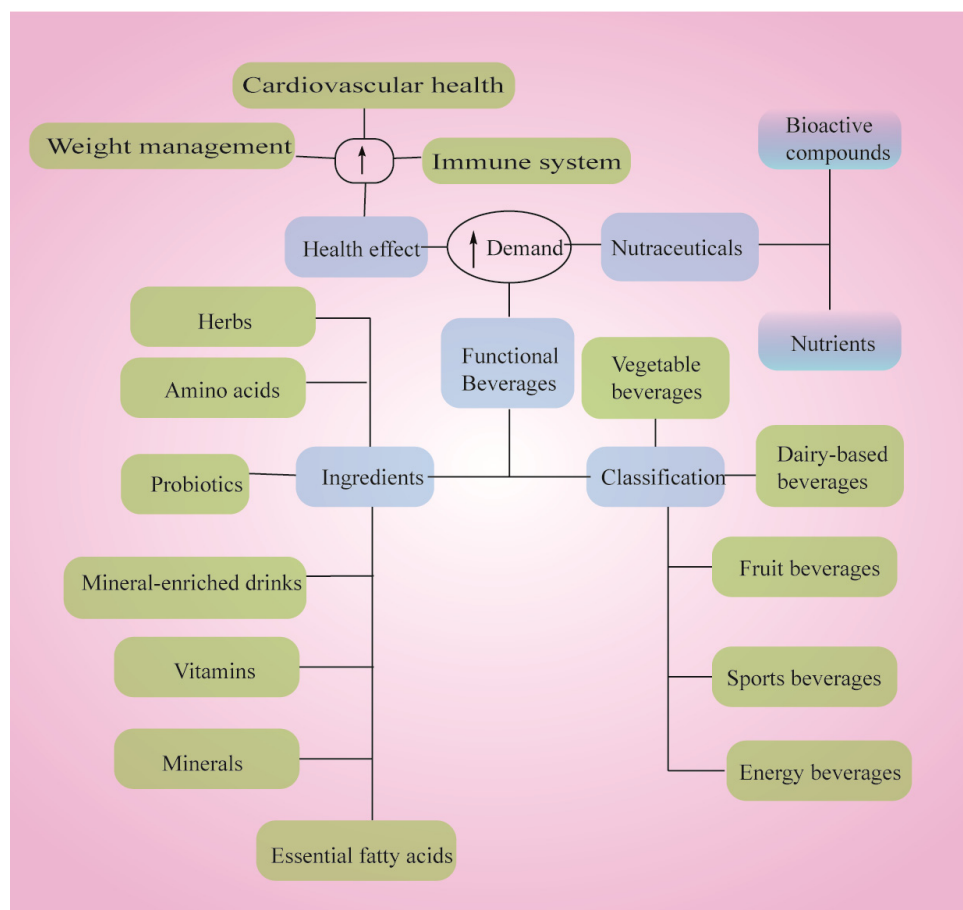
**Abstract:** The growing global interest in functional foods containing nutrients capable of adding possible beneficial health effects is rapidly increasing both interest and consumer demand. In particular, functionalized beverages for their potential positive effect on health e.g., decreasing cholesterol level, lowering sugar, high fiber content, ability to enhance the immune system, and help digestion, have recently received special attention. Among the different beverages available on the market, probiotic dairy and non-dairy products have attracted much attention because of their affordable cost and their numerous therapeutic activities. Fermented milk and yogurt are currently worth €46 billion, with 77% of the market reported in Europe, North America, and Asia. Consumption of dairy beverages has some limitations due for example to lactose intolerance and allergy to milk proteins, thereby leading consumers to use non-dairy beverages such as fruit, grains, and vegetable juices to add probiotics to diet as well as driving the manufacturers to food matrices-based beverages containing probiotic cultures. The purpose of this review article is to evaluate the therapeutic performance and properties of dairy and non-dairy beverages in terms of probiotic, prebiotic, and synbiotic activities.

**Keywords:** beverages; probiotics; health; functional food; prebiotics; synbiotics

## 1. Introduction

Beverages make important contributions to nutritional intake, and their role in health has recently received much attention. Beverages meet consumers' demands in terms of size, shape, storage, and possibility to contain desirable nutrients and bioactive compounds [1]. Recently, Ghoshal et al. [2] discussed beverages as a source of nutraceuticals [3–17], clearing classification, importance, health benefits, interaction with the food, retention during storage, and delivery in the human body. Ingredients of functional beverages include vitamins, minerals, essential fatty acids, amino acids, and herbs. Functional beverages can be useful to support the immune system, improve gut or cardiovascular health, help in weight management, or act as an adjuvant to counteract the aging processes.

Functional beverages can be classified as dairy-based beverages (including probiotics and mineral enriched drinks), vegetable and fruit beverages, and sports and energy drinks [18]. A scheme is shown in Figure 1. Several types of commercial products are available on the market and are identified as sport and energy drinks or as enriched beverages. Nutraceuticals providing specific health benefits as well as drinkable probiotics containing supplements and dietetics drinks are also available on the market [19].



**Figure 1.** Scheme of functional beverages.

Orrù et al. [20] reviewed the current information available in the literature regarding micronutrient-enriched functional beverages in sport taking into account the athletes' health, sports performance, and recovery. Popular sports drinks represent a compromise that meets the needs of most people in several situations, even if no single formulation is able to fulfill all of them. Concerning dairy-based beverages, it is worth mentioning the review of Barbano et al. [21], which examined the last 100-years of the production of a wide range of types of milk available on the market ranging from skim and low-fat milks, to flavored milks, from lactose-reduced milk and long-shelf-life milks, to milks with higher protein and calcium contents. Particular reference has been given to the production technology of high-protein sports beverages. Sun-Waterhouse [22] overviewed the research opportunities in the emerging area of fruit-derived products. Butu and Rodino [23] exploited the synergies between raw materials as ingredients, processing methods, and product formulation as new directions and innovations in the natural vegetable and fruit beverages industry. Chandrasekara and Shahidi [24] described the main evidence concerning herbal beverages: these are prepared starting from natural ingredients belonging to different morphological plant parts, namely leaves, stems, roots, fruits, buds, and flowers. Herbal teas/beverages are rich sources of natural bioactive compounds, such as

carotenoids, phenolic acids, flavonoids, coumarins, alkaloids, polyacetylenes, saponins, and terpenoids, among the others [24].

The demand for fresh and natural eco-sustainable products with appealing sensory characteristics and high nutritional value is exponentially growing, as well as the market demand [25]. Meanwhile, fermented functional foods have attracted much attention for their positive effects on human health [18,25,26]. To this last area belong also foods or beverages with controlled microbial growth and enzymatic reactions, known as fermented foods [27]. The human consumption of fermented beverages, dating back many years as a main part of a daily diet, has formed the fastest-growing segment of the US food market in 2017 (\$1347 billion) [28–30]. Being a low-cost technology, the fermentation plays a relevant role in preserving and enhancing the nutritional value and sensory properties of foods, demonstrating the global importance of fermented beverages containing probiotic bacteria for the human diet [31,32]. There is a wide variety of pre- and pro-biotics, e.g., dairy and non-dairy beverages with high therapeutic efficacy, including cardiovascular system enhancement, weight loss, healthy effect on the digestive tract, boosting immune defense, antioxidant activity, cancer prevention, and improved joint function [33–42]. Accordingly, the present review article has been focused on the evaluation of the positive impact of dairy and non-dairy beverages on human health by considering their probiotic, prebiotic, and synbiotic activities.

## 2. Beneficial Health Effects and Properties of Dairy and Non-Dairy Beverages Containing Probiotics, Prebiotics and Synbiotics: An Updated Shot

The increasing human consumption of probiotic foods can be attributed to the consumers' interest in foods that contain substances beneficial for health [43–45], and this is reflected in the worldwide sales of probiotic foods: global sales rose to \$24.2 billion from \$21.6 billion between 2010 and 2011. It may be estimated that the sales will rise up to \$96 trillion in the year 2020 [46]. This has led food industry researchers to produce new probiotic-containing foods. Therefore, Europe is at the forefront of this market due to the wide range of probiotic foodstuffs produced, such as yogurt, butter, cheese, dairy beverages, ice cream, fruit juices, recovery drinks, and cereal drinks. In fact, sales of these products were observed as \$616.13 million in 2018 at a Compound Annual Growth Rate (CAGR) of 7.7% from 2013 to 2018 [47].

Many of the beneficial effects of such beverages are related to the different types of bacteria, molds, and yeasts known as probiotics, mainly *Bifidobacterium* and *Lactobacillus* ssp. [48–52]. The probiotics are living microorganisms with positive properties for the host's health if consumed in sufficient quantities [52]. The International Scientific Association of Probiotics and Prebiotics has documented that prebiotics are fermented selective substances that result in specific changes in the composition and/or activity of the gastrointestinal microbiota, thereby positively affecting the health of the host in favor of beneficial bacteria constituting the microbiota [53,54]. The synbiotics refers to the combination of prebiotics plus probiotics, which have positive effects on host health through the promotion of beneficial microbial colonization and survival in the host gut [55]. Therefore, synbiotics have been developed to manage some potential problems in the survival of probiotics in the gastrointestinal tract, and they can act as both probiotics and prebiotics [55]. The probiotic cultures can survive in functional drinks mediated by a variety of factors, such as hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), dissolved oxygen (DO), redox potential, pH, and acidity [56]. The probiotics have been reported to have beneficial effects on health [57–59], such as the increased activity of some enzymes, enhancement of intestinal barrier function, generation or induction of antibacterial or bacteriocin-like substances and defensin, effect on host gut microbiota and pathogenic bacteria, alteration of pH, competition for nutrients and physical barriers, modulation of host immune function, intestinal carcinogenesis, cholesterol uptake, and competitive deprivation of pathogenic bacteria [57–59]. In Table 1a–c is reported an updated shot of dairy and non-dairy beverages with prebiotic, probiotics, and synbiotics effects in in vitro, animals and human studies.

**Table 1.** (a) In vitro studies; (b) in animal studies; (c) in human studies.

(a)					
Beverage	Condition	Product	Activity	Effect	Reference
		Kefir	Anti-oxidant activity	Having fermentation observed DPPH radical scavenging, inhibition effect upon linoleic acid autoxidation increased, accordingly, TPC, and inhibition effect upon ascorbate autoxidation boosted	[60]
		Milk and yogurt along with red ginseng	Anti-oxidant and Antigenotoxic effect	The 2,2-diphenyl-1-picrylhy-drazyl radical scavenging activity and oxygen radical absorbance capacity values raised meaningfully in subsequence of adding 2% red ginseng in both.	[61]
Dairy	In vitro	Kefir	Antimicrobial activity	<i>Lactobacillus kefir</i> B6 has been reported having anti-pathogenic activity and being resistant to bile	[62]
		Yogurt and ewe colostrum	Anticancer effects	Antiproliferative effects detected against HT-29 human cancer cells by <i>Lactobacillus plantarum</i> 17C	[63]
		Yogurt	Antioxidant activity	Although <i>Lactobacillus barbarum</i> naturally sweetened the yogurt and increased yogurt aroma, it did not enhance ACE-I prevention activity	[64]
		Kefir	The immunomodulatory effect	The activation of intestinal epithelial cells triggered prevented via flagellin, interleukin-1 $\beta$ , and tumor necrosis factor- $\alpha$ as well as abrogates NF- $\kappa$ B signaling in the cells	[65]
		Papaya juices	Anti-oxidant activity	<i>L. plantarum</i> showed better antioxidant activity than <i>L. acidophilus</i> due to its better oxidation resistance	[66]
		Plant sterol (PS)-enriched beverages	Elevation of phytosterol oxidation products (POPs)	There was more than 75% of total POPs during storage period for three beverages enriched by plant sterol (PS).	[67]
		The fermented pineapple beverage	Sensory and nutritional aspects	Further capacity was observed for <i>Meyerozyma caribbica</i> for general acceptance, aroma and taste on the basis of sensorial findings	[68]
		Cereal beverages	Synbiotic effect	The count of viable probiotics ( $\geq 10^7$ CFU/mL) was found during refrigeration and fermentation for 28 days while maintaining the prebiotic effect.	[69]
Non-Dairy	In vitro	Blueberry beverages	Controlled retinal pigment epithelium (RPE) cells damage	The extracts of blueberry anthocyanin protected the pigmented layer of the retina against damages caused by light through the suppression of apoptosis and aging as well as the down-regulation of vascular endothelial growth factor expression to the normal limit.	[70]
		Apple juice	Antioxidant effect	The analysis of antioxidant properties by DPPH assay in relation to radical scavenging and FRAP functions	[71]
		Fermented beet-root juice	Anticancer and Antibacterial effect	HepG2 as human liver cancer cell line and <i>Listeria monocytogenes</i> controlled	[72]
		Fermented cereal beverages	Antimicrobial effect	Antimicrobial properties were reported for <i>Leuconostoc lactis</i> BT17 to control <i>Enterobacteriaceae</i> .	[73]

Table 1. Cont.

(b)							
Beverage	Condition	Product	Activity	Subjects	Administration Time	Effect	Reference
Dairy	In animal model	Kefir fermented milk	Bifidogenic effect	Animal units were randomized into two groups of 6	Daily administration of 0.75–1 mg of kefir/animal/day in the intervention group (n = 6)	The kefir prebiotic effects on intestinal bacterial populations by increasing through bifidogenic	[74]
		Kefir	Anti-inflammatory in gut	Male Swiss albino mice aged 4 weeks (n = 12)	Daily administration of <i>Kefir</i> (with 108 CFU/mL of <i>L. kefir</i> CIDCA 8348) by the Lk group for 21 days	Down-regulation of proinflammatory mediator expression and anti-inflammatory molecules, which is improved causing an inductive immune system and effector sites in the mouse gut	[75]
		Dairy	Antioxidant effect	The allocation of male rats (n = 24) into three groups, including Group I (control) receiving mere diet, Group II receiving diet plus 5 g/day of common dessert, and Group III receiving diet plus 5 g/day of probiotic dessert	The use of dairy dessert at a dose of 5 g/day containing <i>Lactobacillus acidophilus</i> La-5 at a dose of 8 log CFU/g for 15 days	The treatment caused a reduction in serum lipid profiles by declining LDL, total cholesterol and triacylglycerol	[76]
		Kefir	Cardioprotective effect	The treatment of rats with hypertension maintaining at a temperature of 22 to 23 °C and 12/12-h light/dark cycle	The daily use of kefir at a concentration of 0.3 mL/100 g BW for 60 days	The treatment with kefir caused a reduction in the baroreflex sensitivity and the cardiac autonomic control of heart rate	[77]
		Kefir	Anti-obesity effect	The allocation of mice aged a month into four groups of eight for eight weeks	The use of kefir powder containing 50 mg/kg of polysaccharides, a yeast cell at a density of 102 cfu/g, lactic acid bacteria at a density of 108 cfu/g	The treatment caused a reduction in serum lipid profiles by declining LDL, total cholesterol, and triacylglycerol	[78]
		Kefir	Controlled endothelial dysfunction	The treatment of rats with hypertension aged four months maintaining at a temperature of 22 to 23 °C and 12/12-h light/dark cycle	The use of kefir at a concentration of 0.3 mL/100 g BW for two months	The treatment caused a restoration of intravascular NO availability as well as an elevation of intravascular ROS formation	[79]
Non-dairy	In animal model	whey drinks	Controlled hypercholesterolaemia damage	Male Sprague–Dawley rats (n = 100) kept at a temperature of 23 ± 2 °C, humidity of 55 ± 5% and 12:12 h light-dark cycle	Administration of prebiotic beverages for eight weeks	Fructo oligosaccharides (FOS)-enriched whey drink showed beneficial health effects in the mice with dyslipidemia <i>via</i> the reduction of triacylglycerols (TAG) and low density cholesterol (LDL) levels, and the enhancement of the high density cholesterol (HDL) level	[80]

Table 1. Cont.

(c)							
Beverage	Condition	Product	Activity	Subjects	Administration Time	Effect	Reference
Dairy	In human model	Fermented milk	Controlled infection	Elderly patients (n = 88)	Daily administration of fermented milk beverage once a day for six months	The elderly consumer of <i>L. casei</i> strain <i>Shirota</i> (LcS) -fermented milk- showed improved bowel movements and lower incidence of fever	[81]
		Fermented milk	In type 2 diabetes subjects who are boosted the glycemic control, decrease oxidative stress, and also level of SCFA is regulated	Subjects with type 2 diabetes mellitus (n = 50)	Daily administration of 120 g/d fermented milk for six weeks	Reduce levels of HbA1c and fructosamine, prevented improvement of LDL-C and CT, and reduction of anti-inflammatory cytokines	[82]
		Fermented milk	Controlled type II diabetes	Healthy subjects (n = 17) were randomly allocated to two groups of probiotics (n = 8) and control (n = 9)	Daily administration of LcS-fermented milk drink two times a day for four weeks by the probiotic group	Using up <i>L. casei</i> Shirota [LcS] fermented milk drink impedes high-fat diet-induced insulin resistance	[83]
		Yogurt	CRC survivors having bowel symptoms have been treated and this led to improvement of their life quality	Research units in probiotic Group (n = 28)	Daily administration of probiotics ( $2 \times 10^9$ CFU/ml of <i>L. rhamnosus</i> R0011 plus <i>L. acidophilus</i> R0052) two times a day for 12 weeks	Administration of probiotics meaningfully reduced the percentage of patients suffering from distressing bowel symptom	[84]
		Yogurt	The anti-inflammatory activity	Healthy obese and overweight subjects (n = 75)	Daily administration of 200 g/day of probiotic yoghurt (108 CFU/g of <i>L. acidophilus</i> La5 plus <i>Bifidobacterium</i> BB12, and <i>L. casei</i> DN001) for 8 weeks	A reduction in production of proinflammatory cytokines from PBMCs and in serum hs-CRP levels has been observed in overweight and obese adults	[85]
		Yogurt	Anticancer effect	116 males and 173 females (n = 289)	Different doses of 0 g/day (males and females) in minimal tertile to 85 g/day (males) and 98 g/day (females) in maximal tertile for 12 years	Administration of high-dose supplemented yogurt reduced the colorectal cancer, and stronger protective effect was seen in the male participants than in the females in the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort in Italy	[86]

Table 1. Cont.

(c)							
Beverage	Condition	Product	Activity	Subjects	Administration Time	Effect	Reference
		Fermented dairy product	Shortened duration of respiratory infections	Randomly selected participants with a median age of 76 (n = 1072)	Administration of 200 g/d fermented dairy product (intervention, n = 537) or non-fermented dairy product (control, n = 535) for three months, and then one-month follow-up	The duration of CID, especially URTI and rhinopharyngitis, in elderly subjects was shortened followed by the administration of <i>L. casei</i> DN-114001-containing fermented dairy product	[87]
Non-dairy	In human model	Sugar-sweetened beverages	Anti-obesity effect	Obese and overweight adolescents (n = 224)	Daily administration (12 oz) for a year	Reduced body mass index (−0.57, $p = 0.045$ ) and weight loss (−1.9 kg, $p = 0.04$ ) in the intervention groups were significant when comparing with the controls.	[88]



The dairy industry provides the most popular probiotic-containing beverages due to the effective transport of live probiotics [89–92], which can be associated with various health-promoting effects [93–104]. In a study by Cordeiro et al. [105] a mouse model of infection control caused by *Salmonella typhimurium*, showed that conventional fermented dairy beverages had a protective effect against pathogenic bacteria. Zhang et al. [106] reported antioxidant and gut microbiota regulating activities for the milk fermented by an enhanced probiotic system-containing *Lactobacillus plantarum* YW11. In some other studies, consumption of probiotic yogurt containing *Bifidobacterium lactis* Bb12 caused a significant decrease in *Streptococcus mutans* and *Lactobacillus* isolated from saliva in students with early stages of tooth decay [107]. In a study by Sunarti et al. [108], the use of probiotic-fermented goat milk kefir in rats with type II diabetes, reduced C-reactive protein, fasting glucose levels, HbA1C levels, and serum total cholesterol content [109]. In addition, positive effects have been reported on systemic oxidative stress, systemic inflammation, blood cell damage, and cognitive dysfunction following consumption of synbiotic-supplemented milk fermented with kefir grains [110]. In a study by Jalali et al. [111], the consumption of kefir has been associated with decreased cell proliferation and induction of apoptosis to control human acute erythroleukemia. Kefir administration has also been reported to have ex-vivo anti-inflammatory, anti-metabolic, and antiseptic effects as well as in vitro reduction of cell proliferation, migration and invasion of 4T1 cells [112]. Despite the reports of the positive effects of dairy-based probiotic drinks, they may affect consumer health, e.g., due to lactose malabsorption, allergy to milk proteins, high fat content, and cholesterol content. Therefore, non-dairy fermented beverages have been introduced as substitutes of probiotic dairy beverages in the food industry.

There are traditional non-dairy fermented drinks that are usually produced using fruit or vegetable juice as a main ingredient with many different health-beneficial effects [113–129].

Pomegranate juice fermented with probiotic *L. plantarum* ATCC 14,917 showed a higher total phenolic level and higher antioxidant activity [130]. In a study, Shalgam juice, which contains phenolic and microbial compounds, prevented the growth of Caco-2 cell lines [131]. Yan et al. [132] reported the cholesterol-lowering and antioxidant activity of probiotic fermented blueberry pomace in a mouse model. Łopusiewicz et al. [133] found a high antioxidant activity for kefir-like fermented beverage produced from flaxseed oil cake substrate. Xu et al. [134] reported the attenuation of early-phase after-meal (0–90 min) insulin response by assuming probiotic fruit beverages and also that rose or bilberry juice assumed with the diet in the very early step after a meal (0–30 min) lessened the insulin response and minimized insulin index compared to the control group due to their high phenolic contents. Results of a study recorded at temperatures above 150 °C allowed the observation of the highest antioxidant activity value of fermented grape pomace, which confirms that this activity is not directly related to the polyphenols content in pressurized hot water extraction [135]. Observed antioxidant and cytotoxic activity of *Annona muricata* L. (soursop) pulps and *Rubus glaucus* B. (blackberry) beverages, for human prostate carcinoma cells (PC3) and human cervix carcinoma cells (HeLa), are also another interesting example, since IC<sub>50</sub> values like 0.8460 ± 1.29 and 7.1940 ± 1.06, have been reported, respectively [136].

### 3. Conclusions

Fermented foods account for about 25% of diets in Europe and 60% in developing countries [117]. In the meantime, these beverages have been reported among the most active functional foods. Overall health and disease prevention can be ensured by consuming functional beverages as desirable eating habits. Probiotic beverages as functional foods are currently a specialty in the field of selfcare and complementary proactive medicine. Both dairy and non-dairy beverages have a globalized demand for potential health benefits. Some drawbacks to such products can be overcome by new probiotic strains with the ability to grow and adapt to gastrointestinal conditions and high therapeutic ability by investigating various aspects of these products. Corbo et al. [18] indicated the main direction of functional beverages such as exploitation of microorganism functionality, optimization of the



production and formulation of novel functional beverages, use of prebiotics and synbiotics, use and processing of natural ingredients, and the use of by-products of fruit and food industries to recover and use functional ingredients. The recent work of Rovinaru, and Pasarin, [137] remarked the importance of investigating the functionality of synbiotic microcapsules in protecting the survivability of probiotic cells during processing and storage and underlines challenges for synbiotic formulation in fruit beverages.

Despite the beneficial effect of probiotics on human health, they have shown certain complications, including development of genes related to antibiotic resistance, translocation to blood or tissues, spread of virulence factor in some strains, increased odds ratio of sepsis in preterm newborns and delayed colonization of some normal microflora [138]. In comparison with the probiotics, many studies reported useful efficiencies for postbiotics on host physiological parameters, including certain chemical structure, safety dose factors and prolonged shelf life [139,140]. The probiotics synthesize the postbiotics as non-viable or metabolic byproducts within the fermentation process, whose combination with other nutritional components can show health-promoting effects [141,142]. These compounds include organic acids, short chain fatty acids, endo-polysaccharides, exo-polysaccharides, peptides, cellular superficial proteins, plasmalogens, teichoic acids, muropeptides derived by peptidoglycan, and various enzymes.

As observed by Helkar et al. [143], the search for new functional food ingredients from natural sources and from by-products represent one of the most important challenges in food science and technology. Food industry by-products represent a valuable source of minerals, proteins, fatty acids, fibers, and bioactive substances, and they can constitute as an important raw material for the development of novel functional foods and beverages also. Recently several works remarked how the nanotechnologies open new triggering opportunities and challenges for functional foods development [144–146]. Particularly the current review of Durazzo et al. [147] gives an updated overview on nanoprebiotics and nanoprobiotics.

Grumezescu and Holban [148] presented the impact of novel technologies in nanoengineering on the design of improved and future beverages, with particular regards to nanoencapsulation, the use of metallic nanoparticles and/or nanofibers. Ozdal et al. [149] well described the role of encapsulation in functional beverages. For instance, Tamjidi et al. [150] indicate nanostructured lipid carriers containing hydrophobic nutraceuticals (astaxanthin-loaded nanostructured lipid carrier) have potential to be used for functional beverages/foods development.

At same time, considering how nowadays, consumers expect to be sure that a food product complies with its label and demand further information regarding the origin and sustainability of the product itself, it is of utmost importance to ensure both authenticity and traceability of beverages as recently outlined by Kamiloglu et al. [151].

Despite the many reports on the beneficial effects of probiotics on health, their effects on many diseases have not been fully investigated, and their mechanisms of action for promoting health and controlling disorders need further investigation. Among these, the dose-dependent effect of probiotics is a complex problem that needs to be resolved because short-term consumption of beverages has had a limited impact on the pathological process, and they have complex compounds, different levels of active ingredients, and poor standardization. Another problem is to consider appropriate controls for comparative analysis [152]. Accordingly, the effects of probiotics on health must be proven through the necessary protocols for a systematic method to probiotic evaluation. Pilot and serious screening and evaluation to achieve the positive therapeutic effects of a variety of dairy and non-dairy beverages can ultimately be investigated using cultured cell-based tests, followed by confirmation through *in vitro* and *in vivo* experiments and trials on animals and humans [153]. Nonetheless, some probiotic functions can be attributed to their antioxidant and anti-inflammatory activities. Several randomized controlled trials examined the safety and efficacy of probiotics to prevent and treat some clinical disorders, and a number of meta-analyses and systematic reviews evaluated the results of clinical trials on patients. As a result, there seems to exist a common agreement on the fact that probiotics can

deliver their effects through the gastrointestinal tract and the immune system, and that their effects are interesting, as expected. The challenge of these beverages can be exploited further by adding active compounds in the view to possibly extend their beneficial health properties considering their easy use as it has been proposed also for foodstuff able to vehiculate micro nutrients in the view of a proactive medicine approach for prevention and therapy of health conditions [154] in a new, wider, and open area of research.

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