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Review

Incorporation of Probiotics and Other Functional Ingredients in Dairy Fat-Rich Products: Benefits, Challenges, and Opportunities

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Abstract: This review focuses on the potential opportunities to incorporate functional ingredients like probiotics in the dairy fat-rich matrix to develop functional foods. Many dietary guidelines and the relevant literature have in general indicated many benefits of consuming milk-fat-rich products for the human body. Milk fat contains essential nutrients, including fat-soluble vitamins; short-, medium-, and long-chain with odd and branched chain fatty acids; essential amino acids; and calcium, which are all known for their bioactive properties. In addition, the incorporation of probiotics, which are known for their bioactive properties, could further enhance the products' attributes. However, direct probiotic addition is known to encounter viability challenges during manufacturing and storage. There is thus an opportunity to introduce a value-added range of dairy fat-rich products imparting bioactive and functional benefits. The current review is an attempt to consolidate information in this area and explore further avenues for the value addition of dairy fat-rich products.

Keywords: milk; fat; value-added; bioactive; fatty acids; opportunities; probiotics; encapsulation

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1. Introduction

Milk fat is one of the most valuable components of milk. The separation of cream from milk dates to the late 19th century when farmers kept the cream as a valuable purchase while utilizing the skimmed milk for consumption. Those times have led to the development and innovation of flavor and texture-rich foods from cream. With time, this led to applications such as churning, butter production, and cheese formulation, leading to a product with longer storage stability. Fat is available from different sources, including animal fat and plant-based. However, the composition and nature of milk fat are unique compared to those of other sources. The most common and acceptable forms of milk fat are cream and butter. Cream contains proteins and neutral lipids, including triacylglycerols (97.5%), monoacylglycerols (0.027%), diacylglycerols (0.36%), free fatty acids (0.027%), and polar lipids (0.6%) in the aqueous phase. The triacylglycerol molecule contains three fatty acids and a glycerol backbone esterified at sn-1, sn-2, and sn-3 positions. The most common milk fatty acids are palmitic (26.3–30.4%), oleic (28.7–29.8%), stearic (10.1–14.6%), and myristic (8.7–7.9%) acids [1,2]. The triglycerides have a wide range of melting points, from -50 °C to 80 °C [3]. Milk fat exists as milk fat globules (MFG) distributed in the aqueous phase of milk, containing a protective layer of milk fat globule membrane. It contains many triglycerides with varying fatty-acids positions. Milk fat contains many shorter-chain fatty acids (C4 to C10) that distinguish their critical properties from other fat sources. Although the size of the fat globules ranges from 0.1–10 µ, they are present in large numbers, around 1.5×10^{10} per mL of milk. This number constitutes a large unit surface area of milk fat globules. MFG contains a surface-active tri-layered membrane of 10–20 nm thickness composed of protein, phospholipids, and glycerides, which is known as the milk fat globule membrane (MFGM). The MFGM of milk fats is the most significant component, as it accounts for many characteristic features, such as the emulsification capacity, and serves as a protective layer to shield the fat from lipolysis and oxidation reactions [1,2,4]. MFGM exhibits excellent physical and biological functional properties due to the presence of a many proteins. The proportion of proteins present in the MFGM may range from 25 g/100 g–75 g/100 g depending on the separation and purification techniques. The major MFGM proteins are butyrophilin (BTN), xanthine dehydrogenase/oxidase (XDH/XO), mucin 1 (MUC1), schiff 6/7 (PAS 6/7), adipophilin (ADPH), fatty acid binding protein (FABP), and proteose peptone 3 (PP3) [4]. Another characteristic of MFGM is its ability to repair itself. For example, the shearing mechanism damages the MFGM and globule structure, leading to reduced fat-globule size. This results in the adsorption of milk proteins, including casein, at the interfacial layers, leading to significant changes in the viscosity and other rheological and textural properties of cream [1].

2. General Benefits of Milk Fat

There has been a growing consensus on dairy fat acceptability as a good source of bioactive compounds. Some of the bioactive fatty acids, including butyric acid (4:0) and conjugated linoleic acid (CLA), are exclusive to the dairy fat source, and intake of low-fat or no-fat dairy products could result in deficiency of these bioactive compounds exhibiting health-promoting benefits in the human body [5]. Table 1, below, summarizes the benefits of short-, medium-, and long-chain saturated fatty acids. Short-chain saturated fatty acids are the carbon chain containing two to six atoms, whereas carbon chains containing more than seven and less than twelve are called medium-chain saturated fatty acids. These short-chain and medium-chain saturated fatty acids demonstrate several benefits, including good digestibility, the low tendency to be stored in the adipose tissue, direct hydrolysis from the intestine to the bloodstream yielding quick energy, metabolism regulation, and signaling of the intracellular system [6].

On the other hand, long-chain fatty acids contain more than 12 atoms of carbon that result in fat accumulation in the body (14:0–18:0). They play a significant role in the modulation of metabolic processes in the body. Chains of 14:0 and 16:0 cause protein changes known as N-terminal myristoylation and sidechain palmitoylation that are responsible for signal pathways, oncogenes, eukaryotic proteins, and interactions between protein membranes, respectively [6–8]. Mild intakes of myristic, stearic, and oleic acids have beneficial or neutral effects on human health. However, the intake of palmitic acid remains a concern in the context of cardiovascular disease [7,9].

The next category includes branched-chain saturated fatty acids. They are comprised of four primary fatty acids, including iso-16:0 and 17:0 and anteiso-15:0 and 17:0, and play an essential role in the regulation of gut health, especially for infants and newborns [10]. Besides the colonization and modulation of beneficial microorganisms in the gut microbiome, branched-chain fatty acids exert beneficial effects on chronic diseases, including cancer and inflammation [11].

The last category of saturated fatty acids includes odd-chain saturated fatty acids. The two primary odd-chain fatty acids include pentadecanoic acid (15:0) and heptadecanoic acid (17:0), which are primarily used as dairy-fat biomarkers. They are known to exert beneficial health benefits. However, limited research sources are available for their benefits and mechanisms [6].

The next category is of trans-fatty acids and conjugated linoleic acid. The former group has been reported to have positive and negative effects on the human body. The latter (conjugated linoleic acid) group includes many linoleic acids in the cis-9 cis-12 18:2. They are known for their bioactive activities, including anti-tumor, anti-atherosclerosis, anti-diabetic and anti-obesity [12,13].

Table 1. Benefits of saturated fatty acids.

Type of Saturated Fatty Acids	Benefits	References
Short-chain fatty acid	• Secretion of colonial mucosa; • Enhanced adhesion of probiotics including bifidobacteria and lactobacilli; • Inhibition of pathogens; • Prevention of colon cancer; • Reduction of inflammation.	[14] [15] [16,17]
Medium-chain fatty acid	• Suppression of fat accumulation through thermogenesis and fat oxidation; • Lipid metabolism; • Weight management.	[18,19]
Long-chain fatty acid	• Improved long-chain omega-3 levels; • Increase and decrease of HDL cholesterol and TAG levels, respectively; • Anti-atherogenic activity.	[6]
Branched-chain fatty acid	• Colonization of gut microbiome; • Reduction of necrotizing enterocolitis; • Inhibition of tumor cells.	[6]
Odd-chain fatty acid	• Positive effects on the plasma phospholipids concentration; • Reduction of type 2 diabetes.	[20–22]

3. A Change in Consumer Preference towards Whole Milk Could Provide More Opportunities to Develop Value-Added Dairy Fat-Rich Products

Although milk fat is a natural and rich-quality milk product, some health concerns regarding saturated fatty acids and cholesterol are also reported from time to time. However, the literature generally indicates neutral or some positive benefits of consumption of dairy fat-rich products. Some studies have documented the effect of fat-rich dairy products on cardiovascular diseases. One of the studies found neutral interventions—there were no associations between whole-fat dairy products and cardiovascular diseases among Danish middle-aged men and women. The study suggested that the intake of whole-fat products like yogurt or cheese instead of milk indicates a lower risk of myocardial infarction. This was due to the complex physical structure of milk-fat products and the favorable effects of fat matrices like cheese on metabolic activity [23]. Other studies have also suggested that the effect of the fat dairy matrix is more significant than the fat, casein, and calcium content for metabolic health [24]. Another study reviewed the relationship between butter consumption and all-cause mortality and found no significant association. The review indicated a neutral association of butter consumption with human health [25].

As per the 2015 Dietary Guidelines Advisory Committee (DGAC), replacing saturated animal fats, including dairy fats, with unsaturated fat sources like non-hydrogenated vegetable oils has been generally recommended. However, the DGAC also suggested that the source of saturated fatty acids plays a more significant role in determining its effects on cardiovascular risk. On the other hand, dairy products are complex matrices containing a heterogeneous variety of nutrients, including essential fatty acids, essential amino acids, fat-soluble vitamins, and micronutrients, including calcium. Moreover, dairy fat-rich products like yogurt and cheese are fermented products containing bacterial cultures imparting favorable effects on human health. Butter is a complex matrix that contains saturated fatty acids and could provide the mechanistic benefits that increase HDL-C and lower VLDL-C, along with the nutritional benefits of calcium, fat-soluble vitamins, and medium-branched-chain fatty acids.

Although milk production has varied over the years, per capita consumption of dairy products has increased [26]. The preference for different types of milk, including low-fat milk, reduced-fat milk, and skim milk, have increased over the years. However, preference for milk fat continues due to its appealing organoleptic attributes. Some studies revealed that although 82% women purchased other types of milk, they preferred whole milk due to its desirable sensory properties, including creamy aroma and texture, high viscosity, and mouthfeel [26–28]. A survey evaluated the preference for creaminess in milk-fat products, including sour cream. The authors reported an overall preference for products rich in fat due to their rich creaminess and visual appearance [29].

A recent study determined the effect of milk fat content on consumer preferences. Three categories, including 59 consumers of skim milk, 64 consumers of low-fat milk, and 49 consumers of whole milk, were investigated. The first category preferred 2% skim milk over the 1% one and the low-fat drinkers preferred whole milk over 2% prepared milk. The whole-milk drinkers preferred whole milk over skim-milk preparations; however, no difference was found between the different percentages (2, 4.5, 6.1, and 6.6) used to prepare whole-milk preparations. Overall, the report revealed that milk fat is preferred by all segments of consumers [26].

Considering these factors together, this presents opportunities for the dairy industry to explore a new value-added range of dairy fat-rich products. As per the Digital Journal report, the fat-rich dairy products market is expected to grow at a compound annual growth Rate (CAGR) of 12.5% by 2023 [30]. This could be attributed to several factors, including increasing awareness of the bioactive properties and nutritional value of saturated fatty acids offered by milk fat and increased awareness towards the consumption of wholesome foods.

4. Scope for the Development of Value-Added Dairy Fat-Rich Products

Due to an increase in the consumer understanding of diet and health, there has been an increase in the consumption of functional foods with the incorporation of bioactive and functional ingredients, including probiotics, prebiotics, and fibers. The demand for probiotic functional foods is increasing exponentially due to increased awareness towards maintaining and strengthening immune health, reducing chronic diseases, and a healthy lifestyle [31,32]. The significant spike in probiotic functional foods came during the COVID-19 pandemic due to the demand for gut-boosting food products. Probiotic functional foods, including fermented milk varieties, cheese, ice cream, dairy beverages, buttermilk, breakfast cereals, baby foods, organic beverages, and confectionary products, have been explored [32]. However, there are technical constraints on delivering the minimum probiotic levels. Factors such as strain selection, processing, storage conditions, environmental factors, and food matrix affect the probiotics' survivability in the final product. Many approaches have been reported to maintain the probiotics' survivability. Out of all these, encapsulation is gaining attention as a technique to enhance probiotics survivability during product manufacturing and storage conditions. The incorporation of these functional ingredients with enhanced bioactive characteristics offers a further opportunity to develop probiotic functional foods with a targeted delivery system to diversify the probiotics product portfolio for consumers at a global level.

5. Potential Incorporation of Probiotics and Other Functional Ingredients in the Dairy Fat-Rich Matrices

5.1. Whipped Cream

Whipped cream is one of the most organoleptically acceptable dairy products, with broad applications in the bakery and confectionery. It is a complex oil-in-water emulsion structure, with fat content ranging from 30–40%, containing air cells, fat globules, and proteins. During the whipping mechanism, the partially coalesced fat globules stabilize the air cells at the air–water interface. This whipping mechanism creates a unique foamy colloidal structure responsible for whipped cream's rheological and structural properties. However, this emulsion system is metastable due to mechanisms including coalescence, creaming, phase inversion, and Ostwald ripening. Improving the stability and the physicochemical properties, including rheology, improves the microstructure of whipped cream. Many previous studies have attempted to evaluate the correlation between whipped cream's physicochemical and microstructural properties by studying different factors such as fat content, incorporation of different functional ingredients, and processing conditions [33].

Fat contents greater than 30% introduce a rigid and stable structure. Incorporating ingredients, including emulsifiers and stabilizers, can promote the partial coalescence of fat globules at the air–water interface through an adsorption mechanism. This can further reduce the interfacial tension and improve the structural stability of whipped cream. Many studies have reported the incorporation of modified whey proteins in the whipped cream matrix to improve the stability through their functional and technological properties. The β-lactoglobulin and α-lactalbumin present in whey proteins are mainly responsible for their most functional properties. These proteins, amphiphilic in nature, generate repulsive interactions and improve the viscosity of the aqueous phase by forming viscoelastic layers through adsorption at the interfacial surface. A study reported the effect of modified whey protein, concentrating on the physicochemical properties of low-fat whipped cream. The results reported that whey proteins modified at lower pH values and higher thermal treatments improved the physical properties of whipped cream, including foam stability, viscosity, and firmness. Adding proteins creates interfacial films to stabilize air bubbles that reduce syneresis through increased viscosity. The modified whey proteins further modulated the structure by forming aggregates and showed an appropriate ratio of aggregated whey proteins to native proteins. In either case, appropriate films do not occur, affecting whipped cream's textural and rheological properties. For example, native proteins cannot form effective adsorption interfacial films alone, whereas aggregates alone cannot adsorb at the interfacial surface [34].

Another study also showed improved rheological and microstructural properties by incorporating other protein sources, including rice bran protein (RBP). This plant-based protein exhibits a protein efficiency ratio of 2.0–2.5, a complete amino acid profile, high digestibility, and reasonable functional and technological properties, including high surface hydrophobicity, foaming, and emulsifying properties. With 35% fat content and 3% RBP, the whipped cream showed a maximum consistency index (9.41), and the highest shear viscosity, yield stress, and storage modulus, indicating improved firmness and elasticity. The microstructures also showed decreased voids in the cream, showing improved foaming structure [35]. Another study analyzed the effect of milk fat globule membrane protein (MFGMP) on whipped cream's rheological and textural properties. MFGMP contains surface-active proteins, including mucin 1 (MUC), xanthine oxidase (XO), butyrophilin (BTN), and periodic acid–Schiff (PAS6 and PAS7) exhibiting excellent foaming and emulsification properties. MFGMP works in a mechanism similar to that of whey proteins and caseins. They reduce the interfacial tension through adsorption at the interface surface, stabilizing the colloidal structure of whipped cream. This study also reported the formation of an aggregated structure that entraps more air during the whipping mechanism, leading to increased structural integrity through an increase in particle size. A higher level of MFGMP resulted in improved overrun, partial coalescence, foam stability, and rheological and textural properties, including an increase in storage and loss modulus, with the increase in frequency indicating a strong viscoelastic network [36]. The below-mentioned bioactive ingredients have been reported to impart functional benefits to whipped cream.

5.1.1. Polysaccharides

Tween 60 and 80, lactic acid ester and saturated mono-glycerides, are commonly added to whipped cream. However, they weaken the protein–protein interactions in whipped cream emulsion stabilized through proteins. Many previous studies have introduced polysaccharide stabilizers, including carrageenan, locust bean gum, and basil seed gum, to form a macro-peptide complex that increases the viscosity and provides solid structural properties. The effects of these hetero-polysaccharides on the physicochemical and microstructural properties of whipped cream were studied. The results demonstrated the pseudoplastic behavior of whipped cream due to the thickening effect of these hydrocolloidal molecules. Adding basil seed gum resulted in a pseudo-gel network through the adsorption at the oil–water interface. The strong thickening effect increased the viscosity, resulting in strong rheological and structural properties; however, it showed a negative effect of low-fat content on the overrun and foam stability [33].

5.1.2. Protein–Polysaccharide

Studies nowadays also focus on developing protein–polysaccharide complexes to enhance the oil-in-water emulsion stability. The proteins being excellent in adsorption properties, and polysaccharides being commonly used for their roles in increasing viscosity, can yield a formulation with high emulsification and viscoelastic properties, further improving the functional properties of products like whipped cream. A study showed enhanced physical and textural properties of whipped cream through the incorporation of sodium caseinate–seed gum complex. The study revealed that higher viscosity and firmness at higher concentrations showed reduced overrun. This was due to the formation of viscoelastic adsorbed layers with sodium caseinate, which makes the emulsion sensitive and reduces the capacity to incorporate further air during the whipping mechanism [37].

5.1.3. Others

Other ingredients like modified cellulose, including carboxymethyl cellulose, hydroxymethyl cellulose, and microcrystalline cellulose, also improve the functionality of fat-rich dairy products, including yogurt and whipped cream [38–42]. This electrical field treatment modifies the structure of cellulose containing beta-D-glucopyranose units linked via four glycosidic linkages. The positive results for 1.5% modified cellulose showed significantly increased viscosity and firmness along with overrun and foam stability. This was due to the modified crystallinity of cellulose that improved water-retention capacity, leading to an increase in apparent viscosity [43].

5.2. Butter

Butter is the most widely utilized milk fat product, containing 80% fat and with 16% water content. It is a complex water-in-oil emulsion containing fat in the crystallized form in a continuous phase. The continuous phase comprises water droplets (2.3–10.6 µm), milk fat globules (2.5 µm), and partially disrupted fat globules. This fat network involves crystal interactions held by strong irreversible and weak reversible bonds. There are three main polymorphs of fat crystals in milk fat, including α, β', and β, as per their stability. The least stable polymorph is the α-crystals easily transformed to β' or β upon thermal treatment. The β-crystals form large crystals that result in a granular structure and sandiness defect. Thus, the preference is for this polymorph to be eliminated or transformed into a stable polymorph through chemical manipulations. The crystallization mechanism involves nucleation and growth mechanism. The former is the nuclei formation, occurring either homogeneously or heterogeneously, depending on the surrounding conditions. These interactions are responsible for the characterization of the rheological properties of butter. Other parameters, including the liquid and solid fat ratios, influence the rheological properties. For example, the fat product would be fully liquid without solid fat, whereas the fat product would be hard and brittle without liquid fat. Some of the previous studies have reported the relationship between the rheological and solid fat content of fat-rich products that further helps to decipher the microstructural details of the fat crystal network [3].

The texture is also an essential parameter of butter, as it determines butter's physicochemical and consumer acceptability. Butter's microstructure impacts the overall textural properties of butter, including the size and interfacial parameters of the milk fat globules. Studies showed improved butter texture using sodium caseinate and Tween 80, offering promising results by improving the butter softness by 10–44%. A recent study evaluated the impact of dairy ingredients, including whey proteins, protein concentrates, and milk powders such as buttermilk powder and whole-milk powder. The findings indicated interfacial modifications, resulting in physicochemical properties similar to control butter when treated with buttermilk powder and milk protein concentrates. This proved that dairy-based ingredients could also be employed as natural emulsifiers to make a noticeable modulation in dairy fat-based complex emulsions like butter.

The value-addition of butter by incorporating other functional ingredients like probiotics has been explored. Some studies developed probiotic butter using unencapsulated cells of *Lactobacillus acidophilus* ATCC 4356 and *Bifidobacterium bifidum* ATCC 29521 [44]. The probiotic butter maintained 6 logs_{10}CFU/g for 30 days, providing proof-of-concept of a potential probiotics source other than fermented dairy products. In another recent study, probiotic butter was developed to create high-quality functional food products. The probiotic strains composed of *Lactobacillus casei*, *Lactobacillus plantarum*, and *Lactobacillus paracasei* were added in a ratio of 1:1:1 in pasteurized milk. The butter treated with probiotic and aromatic starter showed significantly higher LAB counts than control, probiotic starter, and aromatic and starter butter variants [45]. A study conducted in our lab standardized a functional and bioactive formulation containing encapsulated probiotic strains *Bifidobacterium animalis* ssp. *lactis* ATCC27536 (BB12) and *Lactobacillus acidophilus* ATCC4356 (LA5) in salted and unsalted butter matrices. The studies showed that the formulation helped to retain the 1:1 ratio of LA5 and BB12 as compared to the control with unencapsulated probiotics. The study provided a proof-of-concept for utilizing butter as a suitable carrier for functional ingredients like probiotics [46].

Butter has good storage stability; however, rancidification is a significant concern because of the growth of psychotropic bacteria causing lipolysis during refrigeration at storage conditions. The oxidation of free fatty acids deteriorates the flavor profile of butter and lowers its nutritional quality. Secondly, the nutritional recommendations have impacted the consumption of fatty milk products containing saturated fatty acids [47]. Many studies have attempted to incorporate functional ingredients, including antioxidants and oilseeds, to improve the overall flavor and nutritional profile. Spices are widely accepted natural bioactive preservatives due to their safety status. A study aimed to evaluate the effect of cinnamon (*Cinnamomum verum*) on the butter's physicochemical and organoleptic properties. The results indicated the positive impact of cinnamon on the bacteriological and physicochemical attributes of butter [48]. Another study investigated the effect of *Thymus haussknechtii* and *Origanum acutidens* essential oils on the stability of butter as a natural antioxidant. Thymus and Origanum, belonging to the *Lamiaceae* family, are spices widely used as food preservatives. They are known for their pharmacological properties, including antimicrobial and antioxidant properties. Mixed results showed that *T. haussknechtii* had higher antioxidant properties, whereas *O. acutidens* showed more effectiveness against coliforms. Sensory properties were affected, as the samples with essential oils incorporation received lower flavor and odor scores. Overall, this study proved the concept of using essential oils as a potential source of antioxidants [49].

5.3. Recombined Dairy Cream (RDC)

Nowadays, recombined dairy cream (RDC) has also gained attention over the last few years due to the short shelf life of natural cream. Like the native cream, RDC is also thermodynamically unstable. Ingredients like proteins, surfactants, and emulsifiers can help stabilize the RDC. RDC utilizes protein concentrates, anhydrous milk fat (AMF), and other ingredients, including stabilizers and emulsifiers. Previous studies have attempted to stabilize the fats using low molecular weight surfactants (LMWS) that facilitate the adsorption of proteins towards the fat globule interfacial surface. A study observed the combination of glycerol monostearate and Tween 80 on the emulsion stability of recombined low-fat dairy cream. The results showed that Tween 80 did not positively impact the emulsion stability. However, 0.40% GMS improved the zeta potential and apparent viscosity, contributing to improved emulsion stability [50]. In the latest study, the efficacy of using tri glycerol monostearate (TGMS) was studied on RDC as an emulsifier. TGMS is a long-chain fatty acid emulsifier used in food applications [51].

The long-chain fatty acids are known to be more effective than shorter ones as they are likely to increase the crystallization temperature that modulates the cream microstructure. The addition of TGMS concentrations from 0–0.9% in RDC resulted in decreases in fat globule diameter and increases in zeta potential values that further caused the increase in viscosity and lower stress at all shear rates, suggesting an increase in the stability of the colloidal structure. The presence of TGMS inhibited the crystallization that positively impacted the cream's partial coalescence and, hence, microstructural stability. In a similar study, the effect of saturated (MAG-S) and unsaturated (MAG-O) parts of monoacylglycerols was studied on RDC. The MAG-S part behaved as a solid that improved the interfacial interaction by promoting the adsorption mechanism. In contrast, the MAG-O remained a liquid that helped increase the crystallization temperature, improving RDC's structural stability [52]. Other studies have widely used bovine milk proteins to stabilize aerated emulsions like whipped cream.

Milk proteins facilitate better emulsion stability, as they reduce the interfacial tension at the O/W interface by forming viscoelastic films around the milk fat globules. A study demonstrated the impact of different caseinates, including micellar casein concentrate (MCC), calcium caseinate (CaC), and sodium caseinate (NaC), on the physicochemical properties (particle size, surface protein concentration, microstructure, viscosity, and crystallization properties) of RDC. The casein dispersions were prepared at different concentrations (0.5, 1.5, and 2.5% *w*/*w*). The results overall showed positive impacts on the rheological and physical properties; however, the effect varied in different cream systems. For example, the results demonstrated superior stability in MCC-added RDC compared to CaC- and NaC-added variants. This was due to the greater steric repulsions at 1.5% and 2.5% concentrations [53]. As for the whipping properties, MCC and CaC showed great whipping stability at almost all concentrations. Another study observed the impact of camel casein and inulin on the physicochemical properties of low-fat camel dairy cream [54]. Both studies reported similar findings, as a 2% concentration of casein showed improved foaming properties, including a decrease in particle size and an increase in apparent viscosity. Another natural surfactant accepted by consumers and classified as generally recognized as safe, lecithin, which is composed of phospholipids, enhances emulsion stability. A study reported the positive impact of soybean lecithin at a concentration of 0.6% on the stability of RDC. The lecithin caused a reduction in droplet size and resulted in protein recovery as the concentration of native proteins increased. However, the results also reported a decrease in zeta-potential values and viscosity. This was due to the microstructural interactions of native proteins and lecithin phospholipids [55].

5.4. Sour Cream

With the rising status of healthy foods, functional foods are the leading category. Functional ingredients like probiotics provide nutritional benefits by improving the gut microbiome. As per the WHO, probiotics are live microorganisms that confer health benefits on the host when administered. Previous studies have documented using fermented dairy matrix carriers for probiotics, delivering promising viable probiotic counts and other immune benefits [56]. Sour cream is a fermented, cultured dairy product manufactured from pasteurized cream containing lactic acid bacteria that sours and thickens the cream. It has broad applications in several products, including stews, salad dressings, cakes, and cookies. However, sour cream may develop certain quality defects, including whey separation and low viscosity. Several studies attempted to incorporate ingredients like hydrocolloids to reduce these defects. A study incorporated galactomannan gums in sour cream to improve the overall physicochemical attributes of the product. Galactomannan gums are extracted from legume seeds and are widely used linear polysaccharides in the food industry. Locust bean gum and guar gum are the mainly used galactomannans due to the high price of other gums, such as fenugreek. The study evaluated the effect of different galactomannan gums on sour cream's rheological and physical–chemical properties. The addition of guar gum significantly improved the rheological parameters (storage and loss modulus) and water-holding capacity of sour cream, further improving the stability by reducing the serum loss [57].

The addition of probiotics alters the fatty acids composition, affecting the physicochemical properties of the cream. The effect of probiotics on sour cream's physicochemical and microbiological properties has been studied [58]. A mixture of probiotics was added, including *Bifidobacterium*, *Lactobacillus casei*, and *Lactobacillus acidophilus*. The study showed that the addition of probiotics not only improved the nutritional value of the product, but also enhanced the overall acceptance of the product by improving the textural and organoleptic properties. However, further investigation of the bacterial interactions between the background microflora and the probiotic strains is required.

6. Benefits of Incorporating Probiotics

6.1. Defining Probiotics

As per the FAO/WHO, probiotics are live microorganisms that, when administered in adequate amounts, confer a health benefit on the host [59]. Elie Metchnikoff introduced the concept of probiotics with consuming fermented milk products in the 1990s. After that, yogurt was the prominent source of probiotics with beneficial health benefits [32].

6.2. Health and Therapeutic Benefits of Probiotics

Probiotics provide several health benefits, including maintaining, protecting, and enhancing the gastrointestinal system against pathogenic bacteria [32]. Probiotic strains offer common properties, including colonization resistance, short-chain fatty acids production, and bacteriocin production, that regulate, normalize, and modulate the intestinal microbial system through the co-exclusion of pathogens [60,61]. Three main mechanisms are responsible for the positive implications of probiotics: cofactors and nutrients production, co-exclusion with pathogens, and host immune response stimulation [62]. Probiotics also offer therapeutic benefits, including preventing diseases related to food allergies, atopic inflammation, constipation, and colon and bladder cancer [32,63]. Previous studies have shown that probiotics were successful in preventing some diseases, including necrotizing enterocolitis, lactose intolerance, ulcerative colitis, infections of *Salmonella*, rheumatoid arthritis, obesity, Alzheimer's disease, and infantile colic [64–70].

6.3. Consumption of Probiotics through Food Products and Supplements

Probiotics could be present in food products or dietary supplements available as capsules, powders, or tablets. Consumption of probiotic organisms through food products is the most popular approach. Probiotic cultures are incorporated, either in freeze-dried or concentrated frozen forms, for Direct Vat industrial applications. The freeze-dried powders typically contain 10^{11} CFU/g or higher viable cells, whereas frozen cultures usually contain levels higher than 10^{10} CFU/g. Traditionally, probiotics have been added to dairy-based products, including fermented beverages and foods, ice cream, cheese, sour cream, and buttermilk, to name a few [32]. Many factors, including the probiotic strain; pH of the medium; presence of hydrogen peroxide, oxygen, lactic, and acetic acids; and storage and processing conditions during product manufacturing, affect the viability of probiotics. Research has supported the suitable application of probiotics in dairy-based matrices. Recent research has also found that fortifying or supplementing dairy products, especially beverages with whey proteins, has a favorable implication for retaining the viability of *L. acidophilus* and *Bifidobacterium*. The higher buffering capacity delays the post-acidification process and release of sulfur amino acid, lowering the redox potential. Several studies have shown the potential of dairy-based beverages as carriers for probiotic bacteria, especially whey-proteins-based ones [31]. A study formulated a fermented whey-based goat beverage containing *Streptococcus thermophilus* TA-40, guava, and partially hydrolyzed galactomannan seeds, which maintained a probiotic viability of more than

7 logs CFU/mL for 21 days [71]. Although the dairy matrix could be considered a potential probiotics carrier, the dairy industry has processing and storage challenges. Processes like heat exposure and homogenization, the addition of antimicrobial substances such as salt during cheese manufacturing, drying processes, and exposure to oxygen during storage might impact the viability of the probiotic. Therefore, the manufacturing of functional food products should consider technological, environmental, and economic factors [32].

6.4. Challenges in Incorporating Probiotics in Food Matrices

6.4.1. Impact of Microbiological Factors Such as Strain Selection, rate, and Proportion of Inoculation on the Probiotic Viability

A wide range of probiotic microorganisms are potential candidates for incorporation into foods; however, lactobacilli and bifidobacteria are the most applied, and commercially available with GRAS status, genera of bacteria. These two species are present in the human intestine, with *Lactobacillus* prominent in the small intestine and *Bifidobacterium* in the large intestine. *Lactobacillus* species survivability is more robust than bifidobacterial, as they are more resistant to conditions such as low pH and milk substrates. Species of other organisms, such as *Lactococcus*, *Saccharomyces*, *Streptococcus*, *Enterococcus*, and *Bacillus*, are also claimed as probiotics in manufacturing functional foods. Several recent researchers have reported the positive implications of these spore-based probiotic strains, including *Bacillus coagulans* [72,73].

6.4.2. Impact of Product Manufacturing, Processing Parameters and Storage Conditions on the Viability of Probiotics

It becomes imperative to maintain the viability of probiotics for effective colonization and interaction with the intestine microbiome to exert its functional properties [63,74]. Several common factors, including oxygen stress, moisture content, pH, storage conditions, and osmotic pressure, affect probiotic viability. Oxygen stress causes metabolite formation, leading to cell death. Low pH conditions make the probiotic cells vulnerable to maintaining the intracellular pH, causing insufficient availability for ATP, and leading to cell death. Three main parameters, processing, food composition, and storage, are significant in maintaining the probiotic concentration. Processing parameters include incubation conditions (time and temperature), heat treatment conditions, cooling conditions (rate and temperature), packaging materials, storage parameters, and time and temperature generally, along with the environmental conditions. For example, processes like freezing and thawing could damage the cell membrane due to differences in osmotic pressures. Factors including storage time, food additives and preservatives, moisture content or water activity, pH, and medium acidity affect the probiotic's survivability during storage time. Additives such as diacetyl, acetoin, nisin, natamycin, lysozyme, nitrite, and artificial flavoring and coloring agents are detrimental to the probiotic's viability. Growth promoters such as whey protein hydrolysates, casein, vitamins, minerals, and antioxidants are known to promote the viability of the probiotic. These substances support the growth by reducing redox potential and increasing protection through the buffering capabilities of whey protein derivatives, further resulting in a decrease in pH [32].

The presence of molecular oxygen is another critical factor affecting the viability of the probiotic. Oxygen causes the death of viable cells through three mechanisms, namely, the production of toxic peroxides, the production of free radicals through the oxidation of fats, and direct toxicity resulting in the death of probiotic microorganisms. Probiotic strains differ in their oxygen sensitivity. For example, *Lactobacillus* strains such as *L. acidophilus* are more resistant to oxygen presence than are bifidobacteria due to the former's anaerobic nature. Oxygen levels can also rise during storage time as oxygen permeates the packaging material. For example, polyethylene permeates air diffusion into food products and could affect the probiotic cells [74]. This relates to the DNA damage phenomenon resulting from lipid oxidation. Therefore, antioxidants, including catechins and oxygen scavengers, are usually added under vacuum conditions to control water activity and oxygen levels [32].

Other factors, such as pH, acidity, and water activity, directly impact the viability of the probiotics during storage. Low pH conditions damage the probiotic cells, resulting in death. The survival pH range for lactobacilli is 3.7 to 4.3, whereas bifidobacteria are less tolerant to pH conditions of less than 4.6. Therefore, strain selection is important for maintaining the viability reflecting the tolerance to bile and acid conditions in the intestine [32].

6.4.3. Impact of Food Composition on the Probiotic's Viability

The food matrix plays a vital role in providing the beneficial effects of probiotics to the host cell. The history of probiotics in food started with yogurt, and it is still the best-known vehicle for probiotics. Yogurt contains *S. thermophilus* and *L. delbrueckii* ssp. *bulgaricus* (LAB) [75]. Other dairy products containing probiotics have also shown significant success over the past few years. Products including ice cream, fermented milk, frozen dairy desserts, whey-based beverages, and buttermilk are some examples of probiotic milk products [32]. Another food matrix, including juice- or fruit-based products, is a complicated supplementation process compared to those for dairy products. The potential reasons include low pH conditions and insufficient quantities of amino acids and peptides for promoting the probiotic's growth metabolism. However, concentration equally plays an important role. Recent research focuses on adding these high nutritional ingredients at optimum concentrations to serve as the prebiotics for the survivability of probiotics [74]. Therefore, with the increasing concern for food safety and quality, increased awareness of intestinal health and well-being has led to an increased research interest in developing advanced probiotic administrative systems for effective intestinal absorption [67].

7. Encapsulation as a Promising Technique to Address the Probiotic Viability Challenge

Encapsulation is one of the most effective techniques for stabilizing probiotics' viability during processing and storage. This technique encloses bioactive substances like probiotics into a miniature capsule for effective delivery at the targeted site by providing a barrier from environmental factors [67,76,77]. However, encapsulation, including micro (3–800 µm) and nano (10–1000 nm) techniques, has been promptly utilized in the pharmaceutical industry. Concern as to the encapsulating materials' safety and quality restrictions has restricted their usage. Considering these factors, microencapsulation is the most promising approach for retaining the viability of probiotics using biopolymeric materials [67]. It offers many benefits, including improved survivability of probiotics from gastrointestinal conditions; protection from external conditions like pH, oxygen, and temperature; controlled release of the bioactive substance; retaining physicochemical and biological properties of probiotics; and a high concentration of viable cells to ensure claimed benefits of probiotics [76]. The micro-encapsulants containing probiotic organisms can either be dry powders or gel-based, with the target being viable cells' ease and long shelf-stability in a product matrix [62].

7.1. Production of Microcapsules Using Extrusion, Emulsion, and Spray-Drying

Probiotic microcapsules are generally produced using three main processes: extrusion, emulsion, and spray drying. Extrusion and emulsion include cross-linkage of the polymer (polysaccharide or protein) after suspension in oil or dropping into the cross-linker, which is usually performed with a syringe and needles. Spray systems have also been utilized (to reduce the size of capsules) [62]. Encapsulation by emulsion can be done through simple methods, including water-in-oil (W/O) and oil-in-water (O/W) or multiple types like water-in-oil-in-water (W/O/W) and oil-in-water-in-oil (O/W/O), by using one or more immiscible phases known as dispersed and continuous phases. These emulsified capsules protect bioactive substances like probiotics. This is a widely accepted encapsulation technique due to its several advantages, including low cost, simplicity, production of very small (<100 µm) capsules, and no requirement of special equipment [62,63]. To further improve the emulsification efficacy, the layer-by-layer (LbL) technique has been demonstrated to provide additional protection to the probiotic microcapsules. Biocompatible carriers, including chitosan- and alginate-based solutions, are used for the additional coating. Research has shown promising results by yielding a better count of probiotics after the in vivo and in vitro studies [63,78]. The study fabricated a formulation containing *Lactobacillus rhamnosus* GG (LGG) in an emulsified microcapsule containing chitosan, poly alginate, and xanthan gum. The results showed a high viability of LGG by a factor of 10^7 CFU higher in the stomach and 10^4 CFU higher in the small intestine. Other studies showed promising results with: different hydrocolloids using Hylon starch with genipin cross-linkage coatings to encapsulate four probiotic strains, including *Lactobacillus casei* (ATCC 39392), *Bifidobacterium bifidum* (ATCC 29521), *Lactobacillus rhamnosus* (ATCC 7469), and *Bifidobacterium adolescentis* (ATCC 15703) [79]; chitosan-alginate coated microcapsules extending the viability of *Bifidobacterium longum* in GIT and high-temperature conditions [80]; and carrageenan coated by skim milk for the viability of *Lactobacillus plantarum* [81]. Different drying processes prepare dry powders encapsulating probiotics, providing a technological advantage to the microcapsules [62,63].

Spray drying is another common technique for industrial applications, as it provides the best economic feasibility. In this technique, the microcapsules are exposed to an atomizer in a drying chamber at a temperature range of 47–200 °C, followed by cyclone separation. During drying, probiotic organisms undergo several stress factors, including thermal, dehydration, shear, osmotic, and oxidative. Several protective agents, including gelatin, gum arabic, and cellulose, have been adopted to build a physical barrier [62,63].

Other drying processes, like freeze drying, are also effective. This technique involves three steps: freezing, primary, and secondary. The formation of ice crystals during the first stage damages the probiotic organisms. A high freezing rate (5 °C/min) is preferred, in order to avoid it [82]. In addition, use of cryoprotective agents could be a promising technique, as it increases the fraction of unfrozen components, thereby giving less osmotic or mechanic stress to the probiotic cells [62,63]. Several studies have reported the positive implications of using freeze-drying after the encapsulation of probiotic organisms by the emulsification process. A study formulated an effective microcapsule containing *Lactobacillus casei* by double emulsification, alginate coating, and freeze-drying techniques [83]. The study showed 97.3% encapsulation efficiency and 98% survival rate while maintaining 7 logs CFU/g at 50 °C and 70 °C for 20 min. Also, it provided good storage stability for up to 17 weeks by maintaining 7 logs CFU/g at refrigerated conditions. Several other studies also reported higher significant good results using freeze-drying as a drying treatment after the encapsulation with emulsification to produce probiotic microcapsules with enhanced storage stability [84,85]. However, it is time-consuming and does not reduce the particle size compared to the other methods, due to the retention of hydrated structure [62].

Another drying technique, fluid bed drying, is a quick treatment and can protect the probiotics or bioactive materials from low pH [62]. One of the studies showed promising results of microencapsulation of probiotics *Saccharomyces boulardii* and *Enterococcus faecium* using emulsification followed by fluidized bed drying techniques [86]. The results exhibited 25% and 40% higher survival rates for *S. boulardii* and *E. faecium*. However, fluid-bed drying requires aerobic environmental conditions which can directly affect the probiotics' viability, as they are anaerobic [62]. Table 2 depicts the advantages and disadvantages of all three techniques.

The selection of a microencapsulating matrix is one of the substantial components due to the diversified conditions of the gastrointestinal tract. Materials with non-cytotoxic and non-antimicrobial properties are preferred to ensure the targeted delivery of probiotics to the host cells. Considering these factors, polysaccharides and protein-based encapsulating materials are typically used, due to their non-cytotoxicity and biodegradability [62].

Table 2. Production of microcapsules using 3 main techniques.

Technique	Advantages	Disadvantages
Extrusion	• Reduces the size of the • microcapsules • Use of spray systems	• Microcapsules size is impacted by the nozzle size • Alginate bead diameter increases with alginate concentration
Emulsion	• Produces very small capsules (<100 microm) • No requirement of special equipment	• Variables, including agitation speed, material, probiotic strain, and organic acid are difficult to standardize
Spray drying	• Economically viable • 6–10 times lower energy consumption as compared to freeze drying	• Thermal stress, dehydration, oxidative stress
Fluid-bed drying	• Very quick • Lower temperatures than the spray-drying • Good survivability at low pH conditions	• Aerobic environment requirement affects the probiotics' viability • Osmotic stress

7.2. Common Encapsulating Materials for the Probiotic's Entrapment

Most encapsulating materials used include polysaccharides, such as k-carrageenan and alginate; other plant derivatives include starch, gum arabic, xanthan, gellan, and animal proteins like whey, casein, and gelatin [77].

7.2.1. Polysaccharides

The most-employed polysaccharides are k-carrageenan and alginate. K-carrageenan is composed of D-galactose-4-sulphate units and 3,6-anhydrous-D-galactose, combined by alternating α 1 $\rightarrow$ 3 and β 1 $\rightarrow$ 4 glycosidic linkages, whereas alginate consists of 1 $\rightarrow$ 4 linked β-(D)-glucuronic (G) and α-(L)-mannuronic (M) acids. The ability of alginate to form a unique "four G residues" structure allows encapsulants to form using an extrusion process. It has shown promising results in encapsulating bacterial strains due to its mild gelling properties, GRAS (generally recognized as safe) status, and absence of toxicity [62,77]. A sizeable body of research has shown that alginate is a potential vehicle for the microencapsulation of probiotic organisms. A study developed a microcapsule containing the probiotic strains *Lactobacillus acidophilus* LA14 and *Bifidobacterium lactis* B107 using alginate and xanthan gum as the primary and gastrointestinal-resistant polymers, employing the extrusion technique. The results showed good survivability of probiotics in simulated gastric fluid and gut medium with bile salts, and storage stability at refrigerated conditions [87]. Other studies also investigated the positive impact of using polysaccharides as the microencapsulating material on the survivability of probiotics [88–90]. Other polysaccharides, including xanthan gum, gum acacia, guar gum, and locust bean gum, have also been exploited as microencapsulating agents for improving the viability of probiotic cells under GIT conditions [88,91].

Recent developments focus on incorporating a protective material into the polysaccharide -based microcapsules for better controlled-release properties. The most used protective material is chitosan, a linear polysaccharide containing glucosamine and N-acetyl glucosamine residues. Studies have shown better results with chitosan owing to the enhanced cationic capability of the encapsulant [62]. Table 3 depicts the significant characteristics of polysaccharide-based encapsulating materials.

Table 3. Advantages and disadvantages of polysaccharide-based encapsulating materials.

Material	Advantages [62,76,92]	Disadvantages [76,77,92]
k-carrageenan	• Enhanced tolerance towards stress conditions in combination with other polysaccharides	• Induces inflammation, intestinal neoplasia • Sensitivity to high temperatures • Inhibitory effect of KCl on probiotics viability
Alginate	• Mild gelling conditions • GRAS status • Lack of toxicity • Protection of bacteria from acidic conditions • Thermotolerance and freeze-drying resistance	• Porous and stable only in pH range 6–9 making it resistant to GIT conditions • CA: Low drug encapsulation efficiency (EE) • Calcium alginate: difficulty in scaling-up process
Pectin	• Widely available	• Expensive
Chitosan	• Ability to form ionic bonds • Biocompatible • Higher swelling sensitivity to pH changes for pH-controlled delivery of probiotics	• Low water solubility
Starch	• Non-digestible in the small intestine • Good thermal stability	• Fermentation results in lowering of pH
Arabic gum	• Low viscosity in the solution, nontoxicity, pH stability, good retention of volatile compounds, ability to stabilize emulsions	• Partial protection against oxygen • Semi-permeable material
Gellan gum	• Acidic, enzyme resistant, swelling at high pH	• Poor mechanical strength, low stability in physiological conditions, high gelling temperature
Gelatin	• Inexpensive as compared to other proteins,	• Animal origin, religious and dietary preferences, high solubility in aqueous systems

7.2.2. Proteins

Proteins have gained popularity over the last decade for probiotic encapsulation. The most-used proteins are casein, whey, albumin, and soy protein. They are usually gelled using enzymatic, chemical, or sol–gel cross-linkages to enhance their mechanical properties [62,76]. This leads to a stronger protein–protein bond with disulfide cross-linkage. This denaturation process causes a controlled release of bioactive substances like probiotics at the targeted site. Animal-derived and plant-derived proteins are mainly used in the microencapsulation of probiotics [76].

Animal-Derived Proteins

Gelatin is the most acceptable water-soluble protein polymer in the food and pharmaceutical industry. Type A and type B gelatins are commonly used. Type A involves acidic pretreatments with pH 4–4.5, whereas type B is from an alkali pretreatment with pH 8 [93,94]. However, their acceptability is limited in society. Also, there are food safety risks associated with some types of gelatins. For example, beef gelatin can cause spongiform encephalopathy or mad cow disease [76]. The second reason is its high solubility in moderate aqueous solutions [95,96]. However, its physicochemical properties can be modified using one or more polymers to encapsulate probiotics. A study formulated a novel micro-encapsulant system consisting of gelatin coated with alginate polymer for improved survivability of *Bifidobacterium adolescentis* 15703T. The results showed better survivability of probiotics (>7 logs CFU/mL) in simulated gastric and intestinal conditions as compared to uncoated gelatin microcapsules < 7 logs (CFU/mL) [97].

Plant Proteins

Due to the rise in plant-protein diets, there has been increased interest in using plant proteins like soy and pea protein as potential probiotic carriers. They serve several benefits, such as abundance, biodegradability, thermal stability, and resistance to oxygen. Some studies have highlighted plant proteins' functional properties for encapsulating bioactive materials in the food and medicine industries [76,98,99].

Milk-Derived Proteins

Casein and whey are common encapsulating agents. However, due to their good physicochemical properties, whey proteins containing beta-lactoglobulin and alpha -lactoalbumin have been widely used in the food industry [76].

Whey Proteins and Its Derivatives as Potential Encapsulating Agents

Whey proteins and their derivatives, including whey protein concentrates, isolates, and hydrolysates, offer numerous nutritional, biological, and functional benefits, as described in Table 4. These properties make whey proteins and their derivatives potential carriers of bioactive compounds like probiotics in the formulation of functional ingredients and food products [100].

Table 4. Properties of whey proteins and their derivatives for a novel formulation.

Health Benefits	Biological Benefits	Bioactive Benefits	Functional Benefits
Control of appetite Exercise recovery Satiety Rich source of amino acids (cysteine, leucine, isoleucine, valine)	Binding of minerals (Zn, Ca), Binding of fatty acids, immunoglobulins, Binding of iron, Protection against intestinal pathogens, Control of acid development in milk, Regulation of muscle protein synthesis, Pre-cursor of glutathione	Antioxidant activity, Antihypertensive activity, Opioid activity, Anti-diabetic activity, Anti-cancer activity, Immunomodulatory activity, Synthesis of muscle protein	Thermal denaturation, Hydration and solubility, Gelation ability, Emulsification property

Besides these benefits, incorporating whey protein hydrolysates into a food matrix is challenging due to its low thermal stability and poor emulsification properties, as well as fouling problems due to the high protein content. Since most of the products undergo thermal treatments like pasteurization to ensure the safety and shelf-stability of products, whey proteins can aggregate, deteriorating the product's characteristics. Several studies have addressed this technical limitation of thermal stability through the conjugation process. Conjugation of whey proteins with carbohydrates can modify physiological and functional properties through a chemical process known as the Maillard reaction. This includes a series of chemical steps leading to the formation of a covalent bond between whey proteins and reducing sugars during the early stages [100]. This process, commonly known as glycation, is a sophisticated process that significantly affects the food product's functional and physiochemical characteristics [101]. Therefore, standardization of the conjugation process with a suitable reducing sugar is necessary.

One of the previous studies conducted in our lab evaluated the effect of conjugation on the whey protein hydrolysates' (WPH) bioactivities, including antimicrobial, antioxidant, and antihypertensive properties. In this study, whey protein hydrolysates with different degrees of hydrolysis were considered in order to evaluate their antimicrobial, antioxidant, and antihypertensive properties. The results exhibited the higher antimicrobial, antioxidant, and antihypertensive activities of WPH 10 [102]. The associated antimicrobial properties are generally attributed to the electrostatic bonding between the peptides and the cell membrane, one which alters the cell morphology, ultimately leading to cell death [103]. The differences in the antioxidant activities of these hydrolysates can be attributed to the differences in exposure of functional sites after hydrolysis, enzyme to substrate (E/S) ratio, pH, temperature, and incubation conditions [98,104]. The higher antihypertensive activity can be due to the hydrolysate's reactive functional groups and hydrophobic amino acid residues.

The WPH conjugated with maltodextrin retained antimicrobial, antioxidant, and antihypertensive activities [102]. This contributed to the release of amino acid groups, including Amadori compounds, during the advanced stages of the glycation process [105]. The significant increase in the antioxidant activity was due to the functionality of the Maillard reaction products that act as electron donors. For example, hydroxyl and pyrrole functional groups, free amino acids groups, and melanoidin significantly enhance the scavenging properties.

Keeping the technicalities mentioned above, including the thermal instability of whey proteins, viability challenges of probiotics, economic feasibility of the encapsulation technique, and bioactive and functional properties of developed conjugated WPH-10-maltodextrin formulation in consideration, the standardized spray-dried conjugated WPH 10-maltodextrin formulation was used as an encapsulating carrier for the entrapment of probiotic cultures, *Bifidobacterium animalis* ssp. *lactis* ATCC27536 and *Lactobacillus acidophilus* ATCC4356. A value-added ingredient of spray-dried conjugated WPH 10-maltodextrin powder containing entrapped probiotics was standardized. The results showed good retention of probiotics' viability, even after the spray-drying process, as compared to the control containing non-conjugated WPH 10 powders, due to the improved thermal stability of the WPH 10 through conjugation with the reducing sugar, maltodextrin. The scanning electron microscopy images of non-conjugated WPH10 powder showed non-porous spheres and some aggregation representing whey proteins' low thermal stability. On the other hand, conjugated WPH10-maltodextrin exhibited a matrix-type structure with a round and porous sphere [106]. Overall, a value-added formulation was standardized with high viable probiotic counts while retaining whey proteins and probiotics' physiochemical and bioactive properties.

8. Conclusions

With the increase in demand for functional and wholesome food products, these value-added products with functional and bioactive benefits can attract a wide range of consumers looking for diet-specific, ingredient-specific, serving-specific, and nutrition-specific ingredients and food products. Many studies have shown promising results with the incorporation of value-added ingredients. However, pilot-scale studies would be required for evaluating the industrial scale feasibility. Protocols, including viability tests, physicochemical tests, and storage stability studies, also might need to be conducted to facilitate the scope of these milk-fat-rich functional foods.

Overall, milk fat matrices could serve as suitable carriers for functional ingredients, including encapsulated probiotics, with minimal effects on the physicochemical characteristics of the matrix, including rheology and textural, sensory, and chemical properties. Combining the bioactive, nutritional, and physicochemical properties of such ingredients could introduce a value-added range of functional foods into the market. These would help diversify the product portfolio for the consumers while expanding the scope of the dairy industry at a global level.

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