Chapter 25

Probiotic Food Products Classes, Types, and Processing

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/51267

1. Introduction

Probiotic as a term is a relatively new word meaning "for life" and it is currently used to describe a group of bacteria when administered in sufficient quantity, confer beneficial effects for humans and animals [1]. The concept of probiotic bacteria is very old, and is associated with the consumption of fermented foods by human beings, for thousands of years. Since ancient times, man has made and eaten probiotic foods. The earliest types of probiotic food were cheeses and milks made by lactic acid bacterial (LAB) and fungal fermentation, and leavened bread fermented by yeasts fermentation [2]. Fermented food's health benefit has also been long known. Hippocrates and other scientists in the early ages had observed that some disorders of the digestive system could be cured by fermented milk, also, Plinius, the Roman historian, stated that fermented milk products can be used for treating gastroenteritis [3].

In the modern ages, the concern to understand the importance and mechanisms of action of probiotic bacteria to exert their beneficial effects has been raised. In the early 1900s, the Russian microbiologist Ilya Mechinikov, Nobel Prize laureate, attributed the good health and longevity of Bulgarian peoples to their high consumption of fermented probiotic foods. He not only identified the health-giving bacteria used to ferment these foods, he also concluded that the general human being's health is function of the balance between beneficial "good" probiotic bacteria and disease-causing "bad" bacteria in human gut [4]. At this time Henry Tissier, a French pediatrician, observed that children with diarrhea had in their stools a low number of bacteria characterized by a peculiar, Y shaped morphology, and these "bifid" bacteria were abundant in healthy children. Also, Tissier found that these bifidobacteria are dominant in the gut flora of breast-fed babies. The isolated bacterium named *Bacillus bifidus*, and was later renamed to the genus *Bifidobacterium*. Accordingly, he suggested that these bacteria could be administered to patients with diarrhea to help restore

a healthy gut flora [2,3]. This claimed effect was due to bifidobacteria displacement of proteolytic bacteria causing the disease. The works of Metchnikoff and Tissier were the first scientific suggestions about the probiotic use of bacteria. However, In 1917, during sever shigellosis outbreak, the German professor Alfred Nissle isolated a nonpathogenic strain of *Escherichia coli* from the feces of a soldier who did not develop enterocolitis. Disorders of the intestinal tract were frequently treated with viable nonpathogenic bacteria to change or replace the intestinal microbiota. The *E. coli* strain Nissle 1917 is one of the few examples of a non-LAB probiotic. It was till 1960s, when the word "probiotic" was first proposed to describe substances produced by microorganisms and promote the growth of other microorganisms [5]. In 1989, Fuller, in order to point out the microbial nature of probiotics, redefined the word as "A live microbial feed supplement which beneficially affects the host animal by improving its intestinal balance" [6,7]. Another definition was proposed by [6] "a viable mono or mixed culture of bacteria which, when applied to animal or man, beneficially affects the host by improving the properties of the indigenous flora". A more recent, but probably not the last definition is "live microorganisms, which when consumed in adequate amounts, confer a health effects on the host beyond inherent basic nutrition [1,7].

As investigations continued in the probiotic field, its concept has been expanded to include bacteria from intestinal origin beside those bacteria isolated from fermented dairy products [8]. Nowadays, probiotic bacteria are available in a variety of food products, dietary supplements [9] and drugs [10]. Food products containing are almost dairy products – fluid milk and yogurt – due to the historical association of LAB with fermented milk. The most frequently used bacteria in these products include the *Lactobacillus* and *Bifidobacterium* species. Recently, new types of food products containing probiotic bacteria started to be introduced into the markets, including nondairy products, such as chocolate, cereals, beverages, fruits and vegetables products. In the near future wide range of nontraditional food products containing probiotic bacteria are expected to be introduced into the markets, as the researches in probiotic products development continue in both scientific and commercial centers around the world.

2. Safety of probiotic bacteria

Safety considerations of probiotic bacteria are of high importance, as most probiotic bacteria are marketed in foodstuffs or feed supplements. The safety of these microbes has been confirmed through a long experience of safe use in food as starter cultures [11-13]. Bacteria such as *Lactobacillus, Leuconostoc,* and *Pediococcus* species have long been involved in food processing throughout human history, and the ingestion of foods containing live dead bacteria, and metabolites of these bacteria has taken place for many centuries [14]. Generally, LAB are classified as generally recognized as safe (GRAS), and there were no reports of any harmful effects from the consumption of these bacteria through the long history of their use in the processing of many foods (i.e. fermented dairy, fermented vegetables …etc.) [15]. In an epidemiological study of lactobacilli bacteremia case reports,

[16] concluded that the increased usage of probiotic products of lactobacilli did not cause any increase in incidence or frequency of bacteremia in Finland. However, it was found that under certain conditions, some lactobacilli strains have been associated with adverse effects, such as rare cases of bacteremia [12]. Ecologically, bifidobacteria are the predominant bacteria in the intestinal tract of breast-fed infants and are believed to contribute to the good health of infants. Until now, the safety of the bifidobacteria has not been questioned, as the reports of a harmful effect of these microbes on the host are very rare.

The concern of probiotic bacteria safety has been raised with the more recent use of intestinal isolates of bacteria delivered in high numbers to severely ill patients. Use of probiotic bacteria in ill persons is restricted to the strains and indications with proven efficacy. A multidisciplinary approach is necessary to assess the toxicological, immunological, gastroenterological, pathological, infectivity, the intrinsic properties of the microbes, virulence factors comprising metabolic activity, and microbiological effects of probiotic strains [1,17]. Conventional toxicology and safety evaluation is not sufficient, since a probiotic is meant to survive and/or grow in human colon in order to benefit humans. Several methods have been developed for evaluation the safety of LAB through the use of in vitro studies, animal studies, and human clinical studies [14]. Also, proposed studies on intrinsic properties and interactions between the host and probiotic bacteria can be used as means to assess the safety of probiotic bacteria [17,18]. Evaluation of the acute, sub-acute and chronic toxicity of ingestion of extremely large quantities of probiotic bacteria should be carried out for all potential strains. Such assessment may not be necessary for strains with established documented use.

Thus, safety considerations of probiotic bacteria should include:

1. Antibiotic resistance profiles.
2. Infectivity in immune-compromised animal models
3. Toxin production: probiotic bacteria must be tested for toxin production. One possible scheme for testing toxin production has been recommended by the EU Scientific Committee on Animal Nutrition.
4. Hemolytic activity.
5. Metabolic activities (D-lactate, bile salt de-conjugation).
6. Genetic and pathological side effects.
7. Epidemiological surveillance of adverse incidents in consumers (post market).

2.1. Antibiotics resistance profiles of probiotic bacteria

Most bacteria, including LAB and probiotic bacteria are resistant to some antibiotics. This resistance may be related to chromosomal, transposon or plasmid located genes [19]. However, data available on situations in which these genetic elements could be transferred is not sufficient, and whether the situation could arise to become a clinical problem is unknown yet. There is a concern over the use of probiotic bacteria that contain specific drug resistance genes in foods. Probiotic bacteria contain transferable drug resistance genes should not be used for human. So, there is an urgent need for the development of

standardized methodology for the assessment of drug resistance profiles in lactobacilli and bifidobacteria. Due to the relevance of this problem, it has been suggested that further research is needed to assess the antibiotic resistance of these bacteria. When dealing with the selection of probiotic strains, it is recommended that probiotic bacteria should not harbor transferable genes encoding resistance to clinically used drugs. Also, research is needed concerning the antibiotic resistance of lactobacilli and bifidobacteria and the potential for transferring genetic elements to other intestinal and/or food borne bacteria. For example, some strains of *Enterococcus* display probiotic properties, but it was found that *Enterococcus* is emerging as an important cause of nosocomial infections and isolates are increasingly vancomycin resistant. Accordingly, *Enterococcus* is not recommended as a probiotic for human use [14].

3. Regulatory issues of probiotic products

As the global probiotic markets are expanding rapidly, the harmonization of national and international regulations and guidelines are becoming extremely important for evaluating the efficacy and safety of probiotic bacteria. Hence, there would always be a possibility of spurious and ineffective probiotic products with false claims being marketed, it becomes important that these products are standardized and fulfill essential prerequisite before being marketed. So far, there is no international harmonization of probiotic product regulations. Depending on the intended use of a probiotic, whether as a food/food ingredient, a dietary supplement, and/or a drug, regulatory requirements differ greatly among different countries [20]. For most countries, if a probiotic is to be used as a drug, then it must undergo the regulatory process as a drug, which is similar to that of any new therapeutic agent. The probiotic drug safety and efficacy for its intended use must be evaluated and approved before marketing. But, if a probiotic is to be used as a dietary supplement, it is considered as foods, and then these products do not need any evaluation or approval before being marketed. However, there is an urgent need for harmonization of these regulatory standards on probiotic bacteria at the international level to ensure the safety and efficacy of probiotic products for their effective utilization in different countries around the world. However, for most countries, probiotic bacteria are regulated under food and dietary supplements because most are taken orally as foods. These are differentiated from drugs in a number of ways, especially with respect to claims. Drug claims include efficacy in the treatment, mitigation or cure of a disease, whereas foods, feed additives and dietary supplements can only make general health claims, such as structure/function claim [21,22]. A 'health claim' is defined as "a statement, which characterizes the effect relationship of any substance to a disease or health-related condition, and these should be based upon well-established scientific evidences from national or international public health bodies. Examples include 'protects against cancer'. A structure/function claim is defined as "a statement of nutritional support that affects the structure or functioning of the human body, or characterizes the mechanism to maintain such structure or function. For example 'supports the immune system' [23,24]. No therapeutic claim or disease-prevention is known to have been approved by the United States, EU, or Canada [23].

3.1. FAO/WHO approach

The Joint FAO/WHO Expert Consultation on Evaluation of Health and Nutritional Properties of Probiotic bacteria has developed and proposed guidelines for evaluating probiotic bacteria in food that could lead to the harmonization of regulations and standards of probiotic bacteria health claims [1,25]. The recommended guidelines included: 1) using a combination of phenotypic and genotypic tests to identify the genus and species of the probiotic strain, as clinical evidences suggested that the health benefits of probiotic bacteria may be strain specific, 2) in vitro testing to delineate the mechanism of the probiotic effect, and 3) substantiation of the clinical health benefit of probiotic agents with human trials. In addition, the manufacturer should take on the responsibility (albeit not required by law) of providing guidance to consumers or clinicians about the type and extent of safety assessments that have been conducted on its products. According to The Joint FAO/WHO Expert Consultation recommendations, even though, that in most countries, only general health claims are allowed on probiotic foods, it is recommended that specific health claims may be allowed on probiotic foods, where sufficient scientific evidence is available. Such specific health claims should be permitted on the label and promotional material.

3.2. United states approach

In the USA, depending on how probiotic bacteria are intended to be used, they may be regulated as a dietary supplement and/or a biological agent. Biological agents require pre-market evaluation of the safety, purity and potency, as well as efficacy for approval by FDA, whereas, dietary supplements do not [25]. According to FDA, the determining factor as to whether a probiotic is a dietary supplement is whether it has been used as a food. A probiotic used for diagnosis, cure, mitigate, treat, or prevent disease is considered as a drug and/or a biological product. FDA's Center for Biologics Evaluation and Research (CBER) regulates probiotic products when used for clinical indications. CBER's Office of Vaccines Research and Review has regulatory jurisdiction over most probiotic products for clinical use [26]. Nevertheless, most probiotic bacteria are regulated as dietary supplements, which were regulated in 1994 by FDA via the Dietary Supplement and Health Education Act (DSHEA). According to DSHEA, probiotic dietary supplements may have a structure/function claim. It is the manufacturer responsibility to notify the FDA before marketing any probiotic product, and determine that the dietary supplements that it manufactures or distributes are safe, and that any claims made about them are substantiated by adequate evidence to show that they are not false or misleading. The manufacturer must also state on the label that the dietary supplement product is not intended to 'diagnose, treat, cure or prevent any disease' because only a drug can legally make such a claim. Unlike Canada and some European countries, the United States has no governmental standards for probiotics. As most probiotic bacteria are claimed to be GRAS, they are not subjected to any specific standards [22]. Currently there are no functional foods are regulated or marketed in the USA, and this is partly because there is no internationally accepted definition of a functional food [24]. The International Food information Council has suggested that

functional foods be defined as foods that provide health benefits beyond basic nutrition [24,25].

3.3. European approach

As in the international level, the different European countries have different national regulations for probiotics. For example in Germany, France, and Italy, the probiotic bacteria in capsule, tablet or powder form have the pharmaceutical products status, whereas, in Denmark, Finland Netherlands, and Sweden, same probiotic products are regulated as food and/or food supplements [24,28]. Food supplements do not require authorities' notification or registration before marketing. In the EU, probiotic bacteria are legally regulated either as 1) foods; for examples, yogurts, dairy drinks, fermented fish, meats & vegetables, and cheeses; 2) food supplements; for examples, tablets, pills, powders, capsules, liquid concentrates in vials, and soft gels; or 3) novel foods. Novel foods are defined as foods/food a ingredient that does not have a significant history of human consumption within the EU countries prior to 15th May 1997 (97/258/EC). According to the novel food regulations, if a probiotic does not have a history of safe use, safety and quality guidelines are laid down. To date, probiotic bacteria for human foods are not governed under specific EU regulatory frame works. Novel Food regulation EU 258/97 is to relevant probiotic in some specific cases. There is therefore a considerable need for harmonization of European legislation on probiotic bacteria considered as food supplements. In contrast with the situation in the USA, even though, that the level of awareness and acceptance of probiotic bacteria in Europe is advanced, neither a legal definition nor specific regulations governing functional foods exist. However, according to Food Supplements Directive 2002/46/EC ''food supplements' are defined as" foodstuffs the purpose of which is to supplement the normal diet and which are concentrated sources of nutrients or other substances with a nutritional or physiological effect, alone or in combination, marketed in dose form, namely forms such as capsules, pastilles, tablets, pills and other similar forms, sachets of powder, ampoules of liquids, drop dispensing bottles, and other similar forms of liquids and powders designed to be taken in measured small unit quantities". Even though, that this directive has generally been elaborated for vitamins and minerals, also, it already states that specific regulations must be laid down for nutrients other than vitamins and minerals. Given that probiotic bacteria fall within this definition of food supplements as used in this Directive, regulation of this kind can help to guarantee the safety and quality of the probiotic products [24].

3.4. Japanese approach

Japan is the only country that have legally defined and regulated functional foods, including probiotics, under the "Foods for Specific Health Use" (FOSHU) system by the Japanese Ministry of Health and Welfare [27]. The FOSHU system allows several health claims for probiotic bacteria include: 1) colonizes the intestines alive, 2) Increases the intestinal beneficial bacteria, 3) Inhibits harmful bacteria, 4) Maintains the balance of the intestinal flora, 5) Maintains the intestines good health, and 6) Promotes the maintenance of a good

intestinal environment [28]. As a result, several probiotic products had received FOSHU approval in Japan [27]. FOSHU system requires the approval of the specific health claim prior to use, and this approval should be based on documented scientific evidences. FOSHU approved products are labeled for the specific health claim. In addition to approved FOSHU foods, many unapproved functional foods are available in Japan. These unapproved foods cannot carry an associated health claim but rely instead on consumer awareness of the probable health benefits of the ingredients [28].

3.5. Canadian approach

Health Canada (HC) and Natural Health Product Directorate (NHPD) which became a law in 2004 are the responsible regulators for food label and health claims in Canada [24,30]. Natural Health Products (NHPs) are considered as a subset of drugs under the Food and Drugs Act, and require assessment and licensing before being marketed. NHPs must be substantiated by sufficient evidence of safety and efficacy under recommended conditions of use, and must be manufactured under Good Manufacturing Practices. For HC/ NHDP, a probiotic is limited to nonpathogenic microorganisms, and is defined "as mono or mixed culture of live micro-organisms that benefit the microbiota indigenous to humans". Foods such as yogurt that contain ''microbes'' are controlled by the Food Products Directorate of HC. As with other food products regulated by HC, probiotic bacteria can carry a structure/function claim, a risk reduction claim, or a treatment claim. The amount and quality of the data to be supplied depend on the claim that is sought. The HC/NHPD regulations concerning probiotic bacteria have requirements related to toxicity and safety [23,31]. It is suggested to use a multidisciplinary approach to assess the pathological, genetic, toxicological, immunological, gastroenterological, and microbiological safety aspects of probiotic strains. Probiotic products in either capsule or liquid form as nutraceuticals' or as functional foods can be found in the marketplace in Canada today. It is not known how many petitions HC has received from companies related to probiotics. However, since its inception in 2004, HC/NHPD has not issued an approved health claim for any probiotic product [30].

4. Labeling requirements

Appropriate labeling and health claims are a pre-requisite for the consumer to make an informed choice. In addition to the general labeling requirements under the food laws of each country, necessary information should also be stated on the label [23,39]. Even though, that currently in most countries, only general health claims are labeled on foods containing probiotics, it is also recommended that specific health claims be allowed relating to the use of probiotics, where sufficient scientific evidence is available [22,25]. For example, the claim that a probiotic 'reduces the incidence and severity of rotavirus diarrhea in infants' would be more informative to the consumer than a general claim that probiotic bacteria' improve gut health'. Such specific health claims should be permitted on the label and promotional material. Also, it is the responsibility of the product manufacturer that an independent third

party review by scientific experts in the field be conducted to establish that health claims are truthfully and not misleading labeled [20].

Hence, the following information must be displayed on the label:

1. Genus, species and strain: To clarify the identity of a probiotic present in food the microbial species must be stated on the label. Genus, species and strain designation should follow the standard international nomenclature. If the selection process has been undertaken, the identity of the strain should also be included since all probiotic effects are strain specific. Strain designation should not mislead consumers about the functionality of the strain.
2. Minimum viable numbers of each probiotic strain at the end of shelf life: The number of probiotic bacteria in food products should be clearly enumerated in order to include them on the label. The label should state the viable concentration of each probiotic present at the end of shelf life. The minimum efficacy level for each probiotic strain that to be maintained till the end of shelf life of product should be scientifically proven.
3. The serving size that delivers the effective dose of probiotic bacteria related to the health claim.
4. An accurate description of the physiological effect, as far as is allowable by law with the required scientific evidence.
5. Proper storage conditions including the temperature at which the product should be stored.
6. Corporate contact details for consumer information.
7. Safety in the conditions of recommended use.
8. Label information must not mislead the consumers to understand that consumption of the food, ingredient or nutrient of such food, can treat, relieve, cure or prevent a disease.

5. Probiotic food products

5.1. Dairy probiotic products

Dairy foods, fermented and non-fermented, have played important roles in the diet of humans worldwide for thousands of years. Since the observations of Mechinikov, in the early 1900s, there has been an increasing interest in the benefits of certain microorganisms; i.e. LAB and probiotic gut flora, and their effect on human general health, body functions, and life longevity. Currently hundreds of probiotic dairy products are manufactured and consumed around the world; typical examples include pasteurized milk, ice-cream, fermented milks, cheeses and baby milk powder [31-35]. The overall pattern of consumption of all types of probiotic dairy products is steadily expanding in the majority of countries in the world. The beneficial health claims are the main reasons behind the popularity and high consuming rates of these products in different communities. Milk is an excellent medium to carry or generate live and active cultured dairy products. The buffering capacity of milk helps to improve the survival of probiotic flora in the GI tract [35]. However, fermented

foods remain the main vehicle to deliver probiotic bacteria [9,36]. Among the fermented milk products, yoghurt is by far the most popular and important vehicle for the delivery of probiotic bacteria [32,37]. Fermented dairy foods are well suited to promoting the positive health image of probiotic bacteria for several reasons: 1) fermented dairy foods already have a positive health image; 2) consumers have the fact that fermented foods contain living microorganisms (starter cultures); and 3) probiotic bacteria used as starter organisms combine the positive images of fermentation and probiotic cultures [38]. In probiotic fermented dairy products, viability of most of probiotic strains are affected as a result of antagonistic interaction between starter cultures and probiotic strains, as well as acid production in these cultured products [31,39]. As a result to these factors, a new trend in producing probiotic non-fermented dairy products has emerged. Wide range of probiotic non-fermented dairy products are produced and marketed by far, such as cheese, ice-cream, and fresh milk [31,33,40].

5.1.1. Fermented milks and yogurt (bio-yoghurt) probiotic products

For the maximum probiotic bacteria viability and optimal therapeutic effects, different types of food products were proposed as a carrier for probiotic bacteria by which consumers can take large amounts of viable probiotic cells. Yogurt, as a fermented milk product, is one of the most popular food carriers for the delivery of probiotic. Yogurt has long been recognized as a product with many desirable effects for consumers, and it is also important that most consumers consider yogurt to be 'healthy', add to that incorporation of probiotic bacteria, such as *L. acidophilus* and *B. bifidum*, into yogurt may add extra nutritional-physiological values [37,38]. Different types of yogurt and yogurt like products are manufactured around the world with different textures, including; natural-set yogurt, stirred yogurt, and drink yogurt, and these products differ greatly in their content of nonfat solids: 16–18%, 13–14%, and 11–12%, respectively [39].

Yogurt is a fermented milk product that has been prepared traditionally by allowing milk to ferment at 42–45°C. Modern yogurt production is a well-controlled process that utilizes ingredients of milk, milk powder, sugar, fruit, flavors, colorings, emulsifiers, stabilizers, and standard pure cultures of LAB (*Streptococcus thermophilus* and *L. bulgaricus*) to conduct the fermentation process. *S. thermophilus* and *L. bulgaricus* exhibit a symbiotic relationship during fermentation process of yogurt, with the ratio between the species changing constantly. The pH of commercial yogurt is usually in the range of 3.7–4.3 [38]. Recently new yogurt products, known as "Bio-Yogurt", have been manufactured by incorporating live probiotic strains in addition to the standard cultures, *S. thermophilus* and *L. bulgaricus*, into yogurt, since the recent discoveries in several aspects of bioscience support the hypothesis that, beyond nutrition, diet may modulate various functions in the body [32]. The Bio-Yogurt products have been formulated with different types of probiotic strains; mainly species of *Lactobacillus* and *Bifidobacteria*; include *L. acidophilus; L. casei; L. gasseri; L. rhamnosus; L. reuteri; B. bifidum; B. animalis; B. infantis; and B. longum* [32,34,35,41-43] Therefore, Bio-Yogurt is a yogurt that contains live probiotic cultures, the presence of which

may give rise to claimed beneficial health effects. Different types of Bio-Yogurts are produced by far, including, plain, stirred, flavored, and fruits added Bio-Yogurts.

For the production of Bio-Yogurt, similar processing procedures of traditional yogurt are applied with the addition of live probiotic starter cultures. Heat treated, homogenized milk with increased protein content (3.6–3.8%) is inoculated with the standard starter cultures at 45◦C and incubated for 3.5-5h [32]. The most common procedures of incorporation probiotic bacteria to Bio-Yogurt include: (1) addition of probiotic bacteria together with standard starter cultures; (2) two-step fermentation, which includes the fermentation of milk first with probiotic cultures to achieve high levels of viable cells, and then addition of standard starter cultures to complete fermentation; (3) two batches fermentation, in which two separate batches of pasteurized milk are fermented, one with probiotic cultures and the other with standard starter cultures, and then the two batches are mixed together; (4) the use of a probiotic alone as a starter culture. In this situation, the time of fermentation is generally higher than regular yogurt production using non-probiotic starter cultures [32,44]. However, the use of the probiotic bacteria alone in the production of yogurt was not sufficient to produce high quality product, where the pH values and the final characteristics (pH values 4.9-5.5, with poor curd formation) of yogurt manufactured by using probiotic species of Lactobacilli and Bifidobacteria, were unsatisfactory [32]. Probiotic bacteria generally tend to exhibit weak growth and acid production in milk, which invariably leads to long fermentation times and poor quality product. This may be due to the sensitive character of the microorganisms in these Bio-products, which adds to the usual difficulties encountered with novel food production (i.e. unusual palatability and consequent limited consumer acceptability) [45]. The poor quality and sensorial characteristics of Bio-Yogurt products are important challenges in probiotic industry [38]. To overcome the problem of the poor quality, two-step fermentation with mixed cultures of the probiotic bacteria and standard starter cultures was suggested. The use of the mixed cultures in the two-step fermentation resulted in yogurt with better acceptability and sensorial quality, and these include longer time for probiotic species to grow and multiply with making use of the traditional cultures to impart the traditional and favorable organoleptic characteristics [45]. Also, it is important to consider the effect of probiotic bacteria addition on the product sensorial characteristics, since metabolites produced by probiotic bacteria may lead to undesirable sensorial effects [46].

Different levels of probiotic bacteria in Bio-Yogurts have been recommended and specified, in order to exert the claimed health effects and considered as probiotic products. The National Yogurt Association (NYA) of the United States specifies that 10^8 cfu/mL of lactic acid bacteria at the time of manufacture, are required to use the NYA'Live and Active Culture' logo on the products containers [47]. In Japan, the Fermented Milks and Lactic Acid Bacteria Beverages Association has specified a minimum of 10^7 cfu/mL of bifidobacteria to be present in fresh dairy products as a standard [48]. Therefore, maintaining the probiotic bacteria viability and survivability during products manufacturing and storage is a very crucial factor for effective probiotic products. Different factors have been found to affect probiotic bacteria viability in Bio-Yogurt products, include, pH, oxygen residues, product

composition, storage temperature, antagonistic activity among probiotic strains and with standard starter cultures. For example, survival of L. acidophilus is affected by the low pH of the yogurt [43], also, the addition of any ingredients, such as fruits or fruits constituents, that lower pH in yogurt may contribute to reduce the survivability of *L. acidophilus* [34]. Rapid loss of viability of *B. animalis subsp. lactis* was reported with increasing percentage of fruit pulp added into yogurt base [49]. Yogurt with high fat content inhibited probiotic cultures, particularly *B. bifidum* BBI [39]. Also, as the probiotic bacteria are oxygen sensitive, oxygen residues in yogurt has an inhibition effect on probiotic bacteria viability [45].

Bio-Yoghurts supplementation with different substances has showed variable effects on probiotic bacteria viability. The supplementation of Bio-Yogurt with ascorbic acid improved the viability of *L. acidophilus* in yoghurts [45]. Oxygen scavenging effect of ascorbic acid is one of the possible mechanisms that may help to improve the viability of probiotic bacteria. Moreover, due to their buffering capacity, the addition of whey protein may enhance the viability of some probiotic bacteria, especially in yogurts with added fruit pulp. Also, the incorporation of prebiotics (indigestible carbohydrates, such as fructooligosaccharides and inulin) [40], and neutraceuticals combination (isoflavones, phytosterols and omega-3-fatty acids) [28, 35] in yoghurt formulations seemed to stimulate the viability and activity of probiotic bacteria. Generally, prebiotics selectively stimulate the growth and activity of probiotic bacteria [20]. It was reported that incubation period, incubation temperature and storage time of yogurts affect probiotic bacteria viability [60]. On the other hand, as a result to oxygen incorporation into yogurts during stirring fruit pulp into yogurt base, stirred-yogurts have lower probiotic bacteria viability levels compared to plain-yogurts. Also, addition of cysteine at 250 and 500 mg/L to yogurt was associated with higher viability of L. acidophilus during manufacture and storage while viability of bifidobacteria was adversely affected by the same levels in different starter cultures, whereas, at level of 50 mg/L bifidobacteria demonstrated better viability. However, in mixed cultures Bio-Yogurt products, antagonistic and symbiotic interactions among probiotic cultures and between probiotic and standard starter cultures are very important factors affecting probiotic bacteria viability. The probiotic cultures must be compatible with each other and with the standard starter cultures, since these micro-organisms could produce inhibitory substances that damage each other and affect probiotic bacteria viability [40,51]. Different pattern of interactions have been demonstrated among different probiotic strains, include, strong, weak, and lack of inhibition [40]. Establishment of suitable combinations of mixed probiotic cultures, to guarantee the maximum probiotic bacteria viability and avoid any inhibitory effect during yogurt manufacturing and storage, requires the assessment of the pattern and the extent of interactions among the probiotic strains and the probiotic strains with the standard starter cultures.

5.1.2. Ice-cream and frozen probiotic products

Ice-cream is a frozen dairy product, consists of a mixture of components, include, milk, flavoring, sweeteners, stabilizers, and emulsifiers agents [52]. Several ice-cream related products, such as plain ice-cream, reduced fat, low fat, nonfat, fruit, and nut ice-creams,

puddings, variegated, mousse, sherbet, frozen yoghurt, besides other frozen products are manufactured and marketed around the world [53]. Smoothness and softness are among the important physical criteria of ice-cream, and these criteria are conferred by vigorous agitation during freezing to incorporate air into frozen product [54]. Ice-cream is a highly appreciated product by people belonging to all age groups, include children, adults, and the elderly public, and by all social levels. Also, the ice-cream low acidity results in increased consumer acceptance, especially by those who prefer mild products.

During the last few decades, new type of the ice-cream products have been introduced to the markets, these products were developed by incorporating probiotic cultures into ice-cream products. The incorporation of probiotic cultures into ice-cream resulted in adding value to the ice-cream product and being considered as a functional product, in addition to being a rich food from the nutritional point of view, containing dairy based material, vitamins and minerals in its composition [33,52]. As a result to the composition/structure, manufacturing procedures, and storage conditions, ice-cream and frozen dairy desserts demonstrated great potential for use as vehicles for probiotic cultures. The ice-cream freezing storage temperature and low risk of temperature abuse during storage has leaded to higher viability of probiotic bacteria [54,56]. The ice-cream composition, which includes milk proteins, fat and lactose, as well as other compounds, make ice-cream a good vehicle for probiotic cultures. Also, ice-cream relatively high pH values (5.5 to 6.5) lead to an increased survival of the probiotic bacteria upon storage. Several studies showed the suitability of ice-creams as a vehicle for probiotic bacteria [33, 53].

The general steps involved in probiotic ice-cream manufacturing are: mixing the ingredients involved (milk, milk powder, sugar, emulsifiers, stabilizers); pasteurizing; cooling to a temperature of around 37–40°C, for the soured ice-cream, the freeze-dried starter cultures (usually yoghurt cultures) and the probiotic cultures is added; subsequent fermentation to a pH of 4.8–5.7, or the addition of a previously fermented inoculums containing both types of lactic cultures; cooling and keeping the mixture at 4°C for 24h for the maturation. Ice-cream mix is produced at this point. The mix is subsequently beaten/frozen, in order to produce the final product, which is packaged and maintained frozen throughout transport, commercial distribution, and storage for consumption. During all these steps after freezing, the temperature of the frozen product should be strictly controlled [33,53].

During probiotic ice-cream development, the ultimate aim of processes optimization is to enhance and maintain the probiotic survivability, so as to guarantee the product functional efficacy [55,56]. This includes the consideration of all the challenges involved in the production of conventional ice-cream. These challenges include: the ingredients microstructure and colloidal properties and/or components used in the formulation; the control of the ice crystallization; the choice of appropriate stabilizers; control of the fat destabilization and the emulsifier functionality [53,55]. Also, the incorporation of probiotic bacteria into an ice-cream products must not affect the product quality criteria, including physico–chemical parameters, such as the melting rate, and the sensory features, which must to be the same or even better than a conventional ice-cream. Ice-cream beaten,

commonly known as overrun, is a process by which the air is incorporated into the product. Overrun is an intrinsic and compulsory step in the ice-cream processing, as it has a crucial impact on the physical properties and sensory acceptance of the ice-cream product, including, body lightness and the formation of a smooth structure, influencing characteristics such as the melt down and hardness properties. In fact, too little air gives the ice-cream a heavy, soggy body while too much air brings a fluffy body [57]. Therefore, overrun is a parameter that should be monitored in ice-cream formulations [58]. The overrun step, as a result to oxygen incorporation into the product, seems to affect the survival of probiotic cultures during processing and storage [33]. However, there is limited information about the effect of the overrun levels adopted during the processing of ice-cream on the survival of probiotic bacteria as well as the sensory acceptance of this kind of product. Recent reports indicated that higher overrun levels negatively influenced probiotic cultures; therefore it was recommended that lower overrun levels should be adopted during the manufacture of ice-cream in order to maintain its probiotic viability through the shelf life [56].

A decrease in the viability of some probiotic species during manufacturing and freezing of probiotic ice-cream was reported as a result to cells damage by freezing and thawing, mechanical stresses of mixing and overrunning during manufacturing and thereby exert a negative effect on functional efficacy of probiotic bacteria in frozen products after ingestion [57]. Addition of inulin and oligofructose demonstrated higher viability of *L. acidophilus* and B. lactis in ice-cream due to prebiotic effect. It is also found that viability of these probiotic bacteria may vary depending on the sugar levels of ice-cream [59,60]. In probiotic ice-cream development, great attention should be given to the other ingredients that are used in the product formulation, especially fruit pulp/juice, which give the product the final flavor. Fruits or their derivatives with a pronounced acidic character should be avoided in ice-creams containing probiotic cultures, since this attribute could influence their sensory acceptance and also decreased the viability of the cultures [61] as its addition decrease pH values. One of the strategies to ensure probiotic bacteria survivability in acidic products is to select acid resistant strains. A recent study suggests the addition of chemical compounds with buffering capacity – carbonate, and citrate salts – at acceptable levels before or during the incubation, in order to eliminate acidic stress [33]. However, fruits and/or flavorings additives with mild and low acidity ought to be used in ice-cream.

5.1.3. Cheese probiotic products

Cheese is the generic name for a group of fermented and non-fermented milk-based dairy products produced and consumed throughout the world in a great diversity of flavors, textures, and forms [62,63]. An essential part of the cheese making process is the curd formation, which involves the conversion of liquid milk into a solid mass that contains casein and fat of the milk. This is achieved by the addition of rennet or acid production by cheese starter cultures to coagulate the casein gel. Curd formation in rennet set cases is carried out through the action of chymosin on the k-casein steric stabilizing layer of the

casein micelle. In cheese making, curd formation is usually followed by several processes such as pressing, salting and ripening. Many cheeses, known as ripened cheeses, need an additional time to ripen under controlled environmental conditions to achieve their own sensory features, particularly flavor and aroma [64]. All cheeses, whether rennet or acid set, can be classified as soft, semi-soft (semi-hard), hard, or very hard cheeses according to moisture contents [63].

As a result to the cultural aspects and the technologies involved with fermented milks and yogurt production, include, relatively short fermentation time, low pH values, oxygen residues, and antagonistic activity of yogurt starter cultures against probiotic bacteria, these cultured products may not be the optimal food carriers for probiotic bacteria to human, as this evidenced by poor probiotic bacteria viability in commercial yogurts [43,51]. In this case, cheese provides a valuable alternative as a food vehicle for probiotic delivery. Cheese high protein content provides probiotic bacteria with a good buffering protection against the high acidic condition in the gastrointestinal tract, and thus enhances probiotic bacteria survival throughout the gastric transit. Moreover, the dense matrix and relatively high fat content of cheese may offer additional protection to probiotic bacteria in the stomach. Also, the relatively high pH values and lack of antagonistic effects of starter cultures, in rennet set cheese may exert optimal conditions to maintain probiotic bacteria viability during cheese making and storage [31]. Accordingly, several soft, semi soft (semi hard), and hard probiotic cheese products have been developed and marketed in the last few years. Jordanian probiotic soft cheese was developed from goat's milk using L. acidophilus and L. reuteri [31]. Cheddar-like cheese was produced by using *B. infantis* [65]; whereas, cheddar cheese was produced by using *L. acidophilus, L. casei, L. paracasei and Bifidobacterium sp*p. [66]. Also, probiotic bacteria of *Bifidobacterium, L. acidophilus* and *L. casei;* and *L. paracasei* A13 were used to produce Argentinian Fresco Cheese, respectively [39,67]. Moreover, it was shown that cheddar cheese is a good carrier to deliver Enterococcus faecium into the gastrointestinal tract of human [68]. Viability of probiotic bacteria during cheese processing and storage is the major challenge associated with the development of probiotic cheese. Probiotic bacteria should be technologically suitable for the incorporation into cheese products so that to retain both viability and functional efficacy during processing on a commercial scale and throughout consumption [69]. Furthermore, from a food processing perspective, it is desirable that such strains are suitable for large-scale industrial cheese production and withstand the processing conditions [70]. With regard to the development of probiotic cheese, this means that such strains should be grown to high cell level before addition into the cheese and/or be able to maintain viability during the manufacturing and/or ripening step [31,64]. In addition, a probiotic cheese should have the same sensory and nutritional qualities as the conventional cheese; the addition of probiotic cultures should not cause any loss in cheese quality. In this context, the level of proteolysis and lipolysis must be the same or even better than cheese which does not have probiotic bacteria [31,66].

Most of the probiotic cheeses have been developed by the addition of probiotic bacteria into cultured cheese [67,71]. In such products, viability of most probiotic strains was affected due to the antagonistic interaction between cheese starter cultures and probiotic bacteria, as well

as acid production in these cultured products [64,69]. Compared to cultured type cheeses and due to its manufacturing process, fresh soft cheese seems to be ideally suited to serve as a carrier for probiotic bacteria as it is an un-ripened cheese, during storage it is submitted to refrigeration temperatures, and its shelf life is rather limited [31]. Fresh soft cheese is a semi-hard cheese and is manufactured in the Middle East and along the shores of the Mediterranean Sea [62]. Most of the soft cheeses are usually made by addition of rennet enzymes to pasteurized milk with no addition of starter cultures. Its pH is almost the same of original milk pH (6.3- 6.5). Moreover, soft cheese is very popular in many parts of world, because of its soft texture and favorable organoleptical characteristics [31]. As a result of these characteristics, soft cheese represents a promising vehicle to deliver probiotic to human. A number of scientific papers reporting the development of fresh cheeses containing recognized and potentially probiotic cultures have been published, which described suitable viable counts and a positive influence on the texture and sensorial properties of these cheeses [31,67]. Method of addition of probiotic bacteria into cheese has a crucial effect in the probiotic viability and functional efficacy during cheese processing and storage. There are two options for the addition of probiotic bacteria during cheese processing. First, probiotic bacteria can be added before the fermentation, together with the starter culture; second, after fermentation. In the first option, the optimal initial inoculum of probiotic to be added and the amount of probiotic which are lost in the whey during its drainage must be evaluated according to the process. In the second option, cheese must be cooled directly after probiotic addition, as metabolic activities of starters and probiotic bacteria are drastically controlled and reduced at these low temperatures. However, other methods for the addition of probiotic bacteria in a semi-hard cheese are the freeze-drying and spray-drying methods. These methods enhanced probiotic viability during cheese processing and storage via the protecting probiotic bacteria against different undesirable conditions encountered cheese processing [72].

Even though there is no specified level of probiotic bacteria in foods that would guarantee the biological activity, but it is increasingly recommended to ingest 10^8 CFU/day [1]. Having in mind that portions of around 100 g of cheese are usually consumed daily, populations of about 10^{6-7} CFU/g lead to an ingestion of 10^{8-9} CFU/daily portion. Addition of prebiotic substances was one of the valuable measures taken to maintain and enhance probiotic viability in cheese products. For example, addition of oligofructose and/or inulin to petit-suisse cheese enhanced the viability of both *L. acidophilus* and *B. animalis* subsp. *Lactis*, while addition of eucalyptus honey reduced the viability level of both probiotic bacteria in the same cheese. The low oligosaccharides content of honey may lead to poor growth and viability reported [73]. Moreover, inulin helps to improve the growth and viability of various probiotic species in a number of different products [50,73].

Also, probiotic bacteria used in food products, such as *Lactobacillus* and *Bifidobacterium* species are: oxygen sensitive or anaerobic; and acid and bile sensitive in nature [74]. Hence, the presence of oxygen, acid and bile may represent a threat for their survival. Several techniques have been applied to enhance and maintain the viability of probiotic bacteria

under harsh conditions typical in cultured dairy products and cheeses, including the selection of probiotic strains tolerant to oxygen, acid and bile, the addition of amino acids and peptides [75]. Another strategy for enhancing bacterial tolerance to stress such as temperature, pH or bile salts is a prior exposure to sub-lethal levels of the given stresses. Stress responses may be used to enhance the survival of probiotic bacteria in stressful conditions and to improve their technological properties [76,77]. Moreover, another alternative for protecting probiotic bacteria to oxygen stress is the use of selected strains of *S. thermophilus* with high oxygen consumption rate as starter for the production of cheeses [75]. Salting of the curd, by immersing it in brine or rubbing salt on the surface is a common step in the manufacture of several varieties of cheeses. In several types of cheeses, specially ripened types, salt is added for preservative and sensorial purposes. However, this slat has an inhibition effect on the growth and the viability of probiotic bacteria in cheese [51]. It is well established that salt level is drastically reduce probiotic viability, especially when salt level is higher than 4% [78]. Therefore, processing of cheeses with high salt content should be optimized to minimize this inhibition effect of slat. Another option is to find ways to protect the probiotic bacteria from the hostile environment. One alternative is micro-encapsulation or cell incubation under sub-lethal conditions [79].

The packaging system is another important factor that is affecting probiotic viability and stability, especially during cheese storage stage. In general, probiotic dairy foods, including cheese, are packaged in plastics films which have different levels of permeability to oxygen. This becomes an important factor because most of the probiotic strains used in food are either oxygen sensitive anaerobic in nature. Therefore, oxygen low permeability plastic films should be used to pack these functional products; alternatively, the practice of adopting other alternatives, such as the use of vacuum packaging can be followed [80].

5.1.4. Kefir

Kefir is a traditional popular beverage consumed for thousands of years in the Central Asia and Middle East countries. It originates in the Caucasus Mountains in Central Asia. Kefir can be considered as natural probiotic fermented milk. It is an acidic-alcoholic fermented milk product, with uniform creamy consistency and a slight sour taste. Milk is fermented with kefir grains, small cluster of micro-organisms held together by a polysaccharide matrix named kefiran, and/or starter cultures prepared from grains [81]. Kefir grains look like pieces of coral or small clumps of cauliflower, which contain a complex mixture of lactic acid bacteria; Lactobacillus, Lactococcus, and Leuconostoc; acetic acid bacteria and yeast mixture [82]. Kefir grains usually contain lactose-fermenting yeasts; *Kluyveromyces lactis, K. marxianus* and *Torula kefir*; as well as non-lactose-fermenting yeasts *Saccharomyces cerevisiae* [81]. Yeasts are important in kefir fermentation because of the production of ethanol and carbon dioxide. *L. kefiri* is the dominant LAB in kefir, comprising about 80% of the LAB flora. The other 20% of the LAB flora in kefir comprises: *L. paracasei subsp. paracasei, L. acidophilus, L. bulgaricus, L. plantarum,* and *L. kefiranofaciens* [83].

The chemical and nutritional composition of kefir is variable and depends on the source and the fat content of milk, the composition of the grains or cultures and the technological process of kefir [84]. Kefir contains vitamins, minerals and essential amino acids that help the body with healing and maintenance functions and also contains easily digestible complete proteins. Kefir is rich in vitamins B1, B12, folic acid, vitamin K, and biotin, as well as calcium, magnesium, and phosphorus, beside essential amino acids such as tryptophan [83,84]. The benefits of consuming kefir in the diet are numerous. Kefir has frequently been claimed to be effective in improving several health and disease conditions, include cancer treatment, intestinal disorders, and promote bowel movement, constipation, flatulence, lactose intolerance [85]. Also, kefir antibacterial, anti-tumor, immunological, and hypocholesterolemic effects have been studied recently, and many reports indicated the efficacy of kefir products in possessing such effects [94,96-97].

Kefir beverages can be made from any type of milk; include, cow, goat or sheep, but commonly used is cow milk. Several substrates are produced in kefir aerobic fermentation includes lactic acid, acetic acid, CO_2 alcohol (ethanol) and aromatic compounds. These substrates provide kefir with its unique sensorial characteristics: fizzy, acid taste, tart and refreshing flavors [83]. There are several methods of kefir production. The traditional and industrial processes are the commonly used methods. The traditional method of making kefir involves the direct addition of kefir grains into milk. The raw milk is boiled and cooled to 20-25°C and inoculated with 2-10% (average of 5%) kefir grain. After 18-24h of fermentation, at 20-25°C, the grains are separated from the milk by filtering with a sieve. Grains can be dried at room temperature and kept at cold temperature to be used in the next inoculation. Kefir milk is cooled before consumption [81, 83]. In the industrial process of kefir, different methods with the same principle are usually applied to produce kefir. The first step is milk homogenization to 8% dry matter, and heating at 90-95°C for 5-10 minutes. Then cooling at 18-24°C and inoculate with 2-8% kefir grains and /or kefir starters in tanks. Fermentation time is 18 to 24h. The coagulum is pumped and distributed in bottles. After maturing either at 12-14°C or 3-10◦C for 24h, kefir is stored at 4°C [81,83].

5.2. Non-dairy probiotic products

As mentioned earlier, dairy products are the main food carriers for probiotic bacteria to human. Limitations of these products such as the presence of allergens, high lactose and cholesterol contents, and the requirement for cold storage facilities have created the need to look for new probiotic product lines based on non-dairy substrates [88, 98]. Furthermore, the increase in the consumer vegetarianism throughout the developed countries generated an increasing demand for the vegetarian probiotic products, as well as the demand for new foods and tastes have initiated a trend in non-dairy probiotic product development [88, 89]. Accordingly, several ranges of non-dairy probiotic products have been developed and marketed in the last two decades. The market available non-dairy probiotic products include: fruits and vegetable, juices, non-dairy beverages, cereal based products, chocolate based products, meat…etc [88, 90-93].

Any new non-dairy probiotic food products should fulfill the consumer's expectancy and demands for the products that are pleasant and healthy; accordingly, the development process would be increasingly challenging [90, 95]. According to [94], new product development is a constant challenge for both scientific and applied research, and it has been observed that food design is essentially a problem of optimization to generate the best formulation. For this purpose, industries need to determine the basic formulation for each product, and the optimum levels of each ingredient to obtain the best sensorial and physicochemical criteria, chemical stability and shelf life, and reasonable price.

5.2.1. Fruits and vegetables probiotic products

Fruits and vegetables are considered healthy foods, as they contain several beneficial nutrients, such as minerals, vitamins, dietary fibers, and antioxidants. Unlike dairy products, fruits and vegetables lack allergens, lactose, and cholesterol, which adversely affect certain segments of the population [96]. Moreover, recent technologies advances have made alterations to some structural characteristics of fruits and vegetables matrices by modifying food components in a controlled way such as pH modification, and fortification of culture media, that might make fruits and vegetables ideal substrates for probiotic bacteria delivery to human [97] Accordingly, several type of probiotic fruits and vegetables products have been developed and marketed, such as fruits and vegetables juices, dried fruits, fermented vegetables, and vegetarian deserts [88,96-98].

As result to their pleasant taste and flavor, as well as acceptability by all age and economic groups, fruit and vegetables juices became one of the most studied, developed and consumed probiotic fruit and vegetable products [96,99,100]. Therefore, it is believed that there is a great potential in developing a new generation of non-dairy probiotic products through successful candidates that are chilled fruit juices and fermented vegetable juices [99,100]. Wide range of probiotic strains, mainly species of *Lactobacillus* and *Bifidobacteria*, such as *L. acidophilus, L. casei, L. paracasei, L. rhamnosus* GG, *L. plantarum, L. fermentum,* and *B. bifidum* have been widely used in the development of many fruit and vegetable products, specially juice products, include orange, pineapple, cranberry, cashew apple, tomato, cabbage, beet and carrot juices. These products have been tested for the suitability as carrier for probiotic bacteria, and the sensory acceptability by the consumer [96,99-101]. In the industrial scale, probiotic bacteria have been incorporated directly and in cell free form into these products. This practice was accompanied with the direct exposure of probiotic bacteria to the acidic conditions of juices and to other unfavorable process conditions, and consequently loose viability. Therefore, a special direct liquid inoculation system, that allows food producers to add the probiotic bacteria directly to the finished product, such as the innovated technology of Tetra Pak's aseptic dosing machine Flex Dos that allows the bacteria to be added to liquids just before they are filled into the cartons, is recommended to overcome the problems of direct inoculation [89]. This innovation is expected to significantly boost the market for the probiotic beverages, which have so far been restricted by the delicate nature of the ingredient and concerns over the contamination. Another challenge encountered the development and marketing of probiotic juices is the juices flavor and

aroma. For example, unpleasant perfumery and dairy aromas, as well as sour and savory flavors have been observed in juices inoculated with *L. plantarum* It has been suggested that the perceptible off-flavors of probiotic orange juice, that often contribute to consumer dissatisfaction, may be masked by adding 10% (v/v) of tropical fruit juices [99].

However, variable patterns of probiotic bacteria viability have been demonstrated in fruit and vegetable juices. It was observed that probiotic's viability in different juices depends on the strains used, the characteristics of the substrate, the oxygen content and the final acidity of the product [45]. For example, when species of *Lactobacillus* and *Bifidobacterium* were added to orange, pineapple and cranberry juices, great differences were observed regarding the acid resistance, and all the strains survived for longer period in orange and pineapple juice compared to cranberry [96]. However, the micro-encapsulation technologies have been successfully applied using various matrices, such as agar, calcium pectate gel, chemically modified chitosan beads and alginates, to provide a physical barrier against unfavorable conditions to protect the probiotic cells from the damage caused by the external environment [100,102]. Vacuum impregnation is another technology applied to improve probiotic bacteria viability in fruit and vegetables products [103]. Using this technology, viability of *L. casei* was improved and sustained in dried apple slices for two months upon storage at room temperature. In this study, dried apple slices were immersed in probiotic cultures grown in liquid, usually natural juices, followed by applying a vacuum pressure of 50 mbar for 10 min, and then atmospheric pressure was restored leaving samples under the liquid for an additional 10 min period [97]. Moreover, fresh apple slices supplemented with *L. rhamnosus* GG was reported to represent a good vehicle for probiotic bacteria, as the probiotic bacteria maintained viability for 10 days at 2-4◦C [104]. Also, fermented table olive represents a potential carrier for delivery of L. paracasei IMPC 2.1 [91].

5.2.2. Cereals and soya probiotic products

Even though, that cereal nutritional quality, compared to milk and meat, is inferior because of their lower protein content, deficiency of certain essential amino acids (lysine), low starch availability, anti-nutrients substances (phytic acid, tannins and polyphenols) and the coarse nature of the grains, cereal grains are still considered as one of the most important food sources of protein, carbohydrates, vitamins, minerals and fiber for large segments of people all over the world [90]. Furthermore, cereal grains are good source of non-digestible carbohydrates that besides promoting several beneficial physiological effects can act as prebiotics that selectively stimulate the growth of *Lactobacilli* and *Bifidobacteria* in the colon [95]. Whole grains are also sources of many beneficial phytochemicals, including phytoestrogens, phenolic compounds, antioxidants, phytic acid and sterols [105].

Usually cereals are consumed either in a fresh or fermented states. There are a wide variety of traditional non-dairy fermented beverages produced around the world, most of them are non-alcoholic cereal beverages [101]. Even though, the non-dairy fermented cereal products have long been created throughout history for human nutrition, it just recently that probiotic characteristics of microorganisms involved in cereal foods fermentation have been

evaluated. Examples of the traditional non-dairy cereal- based fermented beverages include, Boza, Tarhana, Kishk, Chicha, Kisra, Kenkey…etc. [89].

Several studies were carried out to develop probiotic cereal products, especially beverage type. The development of cereal based probiotic products requires the evaluation of the suitability of cereals as growth medium for probiotic bacteria. Probiotic bacteria, especially the strains of *Lactobacillus* and *Bifidobacteria,* have been recognized as complex microorganisms with high nutritional requirement, such as fermentable carbohydrates, amino acids, B vitamins, nucleic acids and minerals [74]. As mentioned earlier, cereals are good source for proteins, carbohydrates, vitamins, and minerals, beside their prebiotic content. These constituents may make cereals a suitable medium for probiotic bacteria growth. Beside that, fermented cereals, as a result to the fermentation process, may have more available nutrients for probiotic bacteria growth, such as improved protein quality and level of lysine, some amino acids may be synthesized, decreased level of carbohydrates as well as some non-digestible poly and oligosaccharides, and increased availability of group B vitamins, optimum pH conditions for enzymatic degradation of phytate and release minerals such as manganese (which is an important growth factor of LAB), iron, zinc and calcium [90]. Therefore fermentation of cereals may represent a cheap way to obtain a rich substrate that sustains the growth of probiotic bacteria. However, in the fermented cereal-based probiotic products, the antimicrobial activity of the LAB of the fermented cereals against added probiotic bacteria must also be considered and evaluated [92].

Several studies have been conducted to evaluate the suitability of different cereal grains to enhance probiotic bacteria growth and maintain their viability [88,92,108]. The oat-based, non-dairy products have been shown to enhance the survival of the probiotic strains *L. reuteri, L. acidophilus* and *B. bifidum*, all of human origin, upon storage at 6°C up to 30 days. These products were fermented by the three strains with and without the commercial yogurt culture. Products fermented in presence of yogurt culture showed lower probiotic bacteria viability compared to product fermented with probiotic bacteria solely. Yosa, a new probiotic oat-based fermented food, similar to flavored yogurt or porridge, contains LAB and bifidobacteria [90]. Yosa is considered as a healthy food due to its content of oat fiber and probiotic LAB, which in combination with the effect of b-glucane might reduce cholesterol and the effect of LAB in maintaining and improving the environment in the intestinal balance of the consumer. Maize, one of the most important sources of food for millions of people, particularly in Latin America and Africa. A maize porridge made of maize flour and barley malt, with high energy density and low viscosity, was fermented with four probiotic strains *L. reuteri, L. acidophilus* (2 strains) and *L. rhamnosus GG*. All strains exhibited a strong growth upon fermentation and storage [88], suggesting that maize porridge supplemented with barely malt is a good medium for probiotic growth. Also, and as a result to the desirable fruity flavors of fermented maize foods, probiotic fermented maize products could have a good world-wide acceptance. Rice is the major cereal in Asia, and its products could be an economical and beneficial medium to develop probiotic foods. The growth of four probiotic bacteria (*L. acidophilus, L. pentosus, L. plantarum* and *L. fermentum*) was found to be higher in germinated rough rice powder (5%, w/w) mixed with

water than in only rice powder with added NaCl. Germinated rice grains found to have an increased content of reducing sugars, total protein and vitamins, mainly B vitamin, which is a very important element required for the growth of *L. plantarum* [74].

Soybean, the most important legume in the traditional Asian diet, is rich in high-quality protein. The products of soybean play an important role in the prevention of chronic diseases such as menopausal disorder, cancer, atherosclerosis, and osteoporosis [107]. Experiments studying the survival of probiotics indicate that soy products, include, soymilk, soy-based yogurt, vegetarian frozen desert, fermented soy tempeh, and soy cheese, are a good substrate for the growth of probiotic bacteria [88,92,106,109]. Soy yogurts were prepared with a yogurt starter in conjunction with either the probiotic bacteria *L. johnsonii*, *L. rhamnosus* GG or human derived *Bifidobacteria*. Probiotic frozen vegetarian soy deserts were developed with the incorporation of *L. acidophilus, L. rhamnosus, L. paracasei* ssp. *paracasei, Saccharomyces boulardii* and *B. lactis* [108]. The neutral pH of the frozen soy dessert improved the probiotic survivability since some probiotic organisms are susceptible to inactivation when stored in acidic conditions [31]. Moreover, it was reported that soymilk fermentation with probiotic bacteria (strains of *Lactobacillus* and *Bifidobacteria*) increased the antioxidative activities of the fermented soymilk, and this further increases the potential of developing a probiotic diet adjunct with probiotic fermented soymilk [88]. Recently, a new probiotic soy based cheese was developed on the basis of Chinese sufu [106]. The soy cheese was made from soymilk fermented with soy cheese bacterial starter cultures and *L. rhamnosus*. The probiotic strain showed good growing pattern during soy cheese fermentation, and good survivability upon storage

5.2.3. Meat probiotic products

Meat is a highly nutritious food with a high degree of nutrients bioavailability and consumers have a high degree of preference for its taste, flavor, and texture. Meat had shown an excellent vehicle for probiotics as a result to meat composition and structure. Furthermore, meat was found to have a protection effect on LAB against the lethal action of bile [109]. One of the most studied and processed probiotic meat products is the dry fermented sausages without heating. Beside the high nutritional value, the characteristics of this type of meat product make it an ideal food matrix for probiotic delivery to human, as, it is a fermented product so the addition of probiotic bacteria will not alter the product sensorial characteristics, also, it is not heat treated, and so the viability of probiotic bacteria will not be reduced. These fermented products are prepared from seasoned, raw meat that is stuffed in casings and is allowed to ferment and mature by LAB starter cultures. The currently commonly employed LAB strains in meat starter cultures include *L. casei, L. curvatus, L. pentosus, L. plantarum, L. sakei, Pediococcus acidilactici* and *P. pentosaceus* [110]. The incorporation of microorganisms that have probiotic criteria is receiving increasing interest. However, few reports so far are available concerning the incorporation of probiotic bacteria into dry fermented sausages. *L. gasseri* JCM1131 has been demonstrated to be useful as a potential probiotic strain for application in meat fermentation and improving its safety

[111]. The efficacy of *L. rhamnosus* FERM P-15120 and *L. paracasei* subsp. *paracasei* FERM P-15121 has also been reported, as potential probiotics in meat products [112]. A mixed culture of the traditional starter culture and a potential probiotic culture of *L. casei* LC-01 or *B. lactis* Bb-12 have been successfully employed in sausage production [113].

The importance of using probiotic bacteria from the meat dominant strains supports the demand for higher numbers of viable cells at the time of consumption, which is a prerequisite for the probiotic to insure beneficial effects on the host. Furthermore, the use of a probiotic starter culture would prove superior in providing more safety, taste and health benefits, as compared to the traditional cultures [114]. LAB strains, include *L. acidophilus, L. crispatus, L. amylovorus, L. gallinarum, L. gasseri,* and *L. johnsonii*, were found to be suitable for meat fermentation and to enhance product safety [111]. Also, it has been reported that the selection of *L. plantarum* and *L. pentosus* isolated from Scandinavian-type fermented sausage as a promising probiotic meat starter cultures [121]. Moreover, *L. plantarum* and *L. curvatus* strains isolated from Greek dry-fermented sausages were resistant to 0.3% bile salts [116].

Various studies have shown that probiotic organisms survive poorly in fermented foods [117]. Nonetheless, probiotic organisms may be encapsulated by the sausage matrix consisting of meat and fat. Alginate-microencapsulated probiotics (*L. reuteri* and *B. longum*) may be an option in the formulation of fermented meat products such as sausages with viable health-promoting bacteria; nevertheless, their inhibitory action against some pathogen organisms could be reduced [118]. *B. longum* and *L. reuteri* encapsulated in Alginate were a suitable option for this purpose. Recently, *B. longum* was successfully protected in-vivo and in-vitro by encapsulation in innovated encapsulation material of succinylated β-lactoglobulin tablets [119].

5.2.4. Chocolate probiotic products

Chocolate is one of the most popular products all over the world, due to its delicious taste and flavor, high nutritious energy, fast metabolism and good digestibility. The presence of cocoa butter, milk and milk based materials, as well as sugar in its composition can be the warranty of an appropriate ingestion of proteins, carbohydrates, fats, minerals and vitamins [120]. Chocolate in its original form has long been known to lift mood, increase mental activity, to control appetite, and improve heart health. However, the high sugar content of conventional brands has raised concerns that their consumption is contributing to the current obesity epidemic, to osteoporosis development in older women, and the raising diabetes incidence in the Western industrialized nations. Nowadays, one of the most important trends in chocolate manufacturing is originated by the consumers' demand of functional or health-promoting chocolate, i.e., chocolate that not only do not adversely consumer health, but also remedy or prevent illnesses such as heart disease, osteoporosis, cancer, diabetes…etc. [121,122] Chocolate itself is a functional food, as it contains sufficient polyphenolic antioxidants and flavonoids compounds. These beneficial compounds in chocolate have been attributed to chocolate health beneficial effects. However, it is now

possible for manufacturers to create functional foods by fortifying and enhancing their products to give them added health benefits have never been possible before, by incorporating probiotic bacteria to chocolate products [120] Developing a Probiotic chocolate product that is affordable and also nutritional for many more people is a challenge. The application of probiotic bacteria into chocolate could offer a good alternative to common dairy products, and allow broadening the health claims of chocolate based food products. Indeed, recent market research on functional food has shown that, in relation to chocolate, digestive health was one of the most important drivers of consumer acceptance [122,123].

The development of probiotic-containing chocolate involves a good understanding of the selected probiotic strains, the chocolate manufacturing process and the different critical points of the process for probiotic survival, as well as the application of specific protective technology [123]. Viability of probiotic bacteria in a product at the point of consumption is an important consideration for the efficacy, as they have to survive during the processing and shelf life of food and supplements, transit through high acidic conditions of the stomach and enzymes and bile salts in the small intestine [95]. Moreover, the sensorial acceptability of the product from the consumer is another limiting factor that determines the success of the product [124]. A few numbers of attempts were made to develop probiotic chocolate products so far. Recently, a chocolate mousse was developed by using probiotic and prebiotic ingredients. Probiotic and synbiotic chocolate mousses were supplemented with *L. paracasei* subsp. *paracasei* LBC 82, solely or together with the prebiotic ingredient inulin [122] It was shown that the chocolate mousse was an excellent vehicle for the delivery of *L. paracasei*, as it enhanced probiotic bacteria growth and viability during chocolate mousse processing and shelf life, and the prebiotic ingredient inulin did not interfere in its viability, as well as the addition of the probiotic microorganism and of the prebiotic ingredient did not interfere in the sensorial preference of the product. Moreover, another chocolate product was evaluated to support the growth and survivability of *L. rhamnosus* IMC 501 and *L. paracasei* IMC 502 mixed 1:1 (SYNBIO). The survival and viability of probiotics were determined during the product processing and shelf-life. The values of viable probiotic bacteria showed that this product could represent an ideal vehicle for probiotic bacteria to human [123].Furthermore, a chocolate product has been evaluated as a potential protective carrier for oral delivery of a microencapsulated mixture of *L. helveticus* CNCM I-1722 and *B. longum* CNCMI-3470 [124], the data in this study indicated that the coating of the probiotics in chocolate is an excellent solution to protect them from environmental stress conditions and for optimal delivery.

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