



Recent developments of non-dairy based probiotics - A mini review

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Abstract: Now a days, probiotic enriched functional food has a growing attention due to its health benefits. Probiotics are healthy live microorganisms that are readily available in human gut system in minor quantity. Intake of adequate amount of probiotics provides beneficial effect on the human health. Even though probiotic products are commonly marketed in the form of fermented dairy products, consumer awareness has recently grown towards the non-dairy based probiotic products basically due to increase of lactose intolerance, vegetarianism and the ability of non-dairy based matrixes to maintenance the health. Therefore consumers are looking for healthy probiotic counterparts by replacing dairy bases. *Lactobacillus spp* and *Bifidobacterium spp* are the predominant and frequently use probiotic microorganism species for formulating non-dairy based probiotic food matrixes. Due to specific flavour, texture, refreshing nature and gut stability, the demand for non-dairy based probiotic products has rapidly increased. Therefore, focus of the development of non-dairy based probiotics including vegetables, fruits, cereals, meat, bakery produces and confectioneries are crucial. The present paper reviews the potential applications of non-dairy base food matrixes as probiotic counterparts and the characteristics that enable the use of these food matrixes as potential carriers of probiotic microorganisms.

Index Terms: Non-dairy, Probiotics, vegetable, meat, bakery products

1 INTRODUCTION

History of the utilization of probiotics is stretched back in to a time before microbes were discovered [1]. Now a days, the beneficial effect of food along with added microbes (like probiotics) are being increasingly promoted by health professionals over the world [2]. Probiotics are bacteria and yeast who have an ability to prevent and/or treat some illnesses through stabilizing the microbial community at the presence in adequate amount [3,4]. Lactic acid bacteria such as *Lactobacillus spp*, *Streptococcus spp*, *Bifidobacterium spp* are the most common probiotic microorganisms that provide beneficial effect to the body [5]. Intake of probiotics incorporated food is one way of obtaining probiotics through oral consumption. The probiotic fortified products are considered as functional foods due to its ability of providing additional important functions for human health [6]. Dairy products are the main category of food stuff which could formulate, transfer and deliver the probiotic bacteria due to availability of significant amount of sugar (e.g. lactose) which provide a medium to grow the probiotic bacteria [4,7–9]. However, milk allergies, increase of lactose intolerance, environmental concerns and problems occur due to diets rich in cholesterol encourage demand for non-dairy based product [10]. The increase preference of new taste and flavor build a motivation and consumer demand for the non-dairy based probiotics further [11,12]. Therefore non-dairy based juices, vegetables and cereal based products, bakery products have been processed to accomplish the increasing consumer demand [13–16].

Fruits and vegetables are healthy foods as same as the dairy, due to contain of lots of beneficial nutrients such as mineral, vitamin, dietary fibers and antioxidants; meanwhile it shows lack of allergen, less of lactose and cholesterol [17]. Characteristics such as intercellular space (pores) in plant tissues, lenticels and tissue lesions in plant cut, damaged vegetable surfaces and minimally processing vegetables (peeled and cut) promote release of cellular content rich in minerals, sugar, vitamins and other nutrients and allow the probiotic bacteria to access the fruit and vegetable products [18]. Further, complex carbohydrates and phenolic like plant compounds may be act synergistically with probiotics [19,20]. However, the ability of probiotic strain to survive during food processing and/or the ability to compete with metabolically active microorganisms occurring in the food matrix are the main concerns which efficacy of a probiotic food is mainly depend on [21]. There are lots of non-dairy based products have been developing over the world under different categories including fruit and vegetable based products, cereal based products, soy based products, baked products, confectioneries, meat based products in addition to dairy based products.

Functional foods and beverages are fortified with addition of exogeneous functional compounds or biogenic producible microorganisms or probiotic microorganisms [22]. However, incorporation of probiotic culture in non-dairy based food is a great challenge due to different reasons. Especially, probiotic viability in food matrix is basically depend on different factors such as PH, temperature, oxygen level and presence of competing microorganisms and inhibitors [23]. Namely Acidity/PH, oxygen level, lack of nutrients and presence of antimicrobial substances in product are directly effect on viability of probiotic cultures within the product [24]. Therefore, prior to develop the probiotic enrich non-dairy products, proper studies should be intended on each and every food category (cereals, soy, fruits, vegetables, meat) [25].

Table 01: Proved microorganisms considered as probiotics [26–28]

| Lactobacillus | Bifidobacterium | Other lactic acid bacteria | Non-lactic acid bacteria |
|--|-------------------------------------|------------------------------------|---|
| <i>Lactobacillus acidophilus</i> | <i>Bifidobacterium adolescentis</i> | <i>Enterococcus faecalis</i> | <i>Bacillus cereus var. toyoi</i> |
| <i>Lactobacillus amylovorus</i> | <i>Bifidobacterium animalis</i> | <i>Enterococcus faecium</i> | <i>Enterococcus faecalis</i> |
| <i>Lactobacillus brevis</i> | <i>Bifidobacterium adolescentis</i> | <i>Lactococcus lactis</i> | <i>Enterococcus faecium</i> |
| <i>Lactobacillus casei</i> | <i>Bifidobacterium bifidum</i> | <i>Leuconostoc mesenteroides</i> | <i>Escherichia coli Nissle</i> |
| <i>Lactobacillus cellobiosus</i> | <i>Bifidobacterium breve</i> | <i>Pediococcus acidolactici</i> | <i>Lactococcuslactis subsp. lactis</i> |
| <i>Lactobacillus crispatus</i> | <i>Bifidobacterium infantis</i> | <i>Streptococcus thermophilus</i> | <i>Leuoconostocmes enteroides</i> |
| <i>Lactobacillus delbrueckii subsp. bulgaricus</i> | <i>Bifidobacterium lactis</i> | <i>Sporolactobacillus inulinus</i> | <i>Propionibacterium freudenreichii</i> |
| <i>Lactobacillus fermentum</i> | <i>Bifidobacterium longum</i> | | <i>Pediococcus acidilactici</i> |

| | | |
|---------------------------------|-------------------------------------|---------------------------------|
| <i>Lactobacillus gallinarum</i> | <i>Bifidobacterium thermophilum</i> | <i>Saccharomyces boulardii</i> |
| <i>Lactobacillus gasseri</i> | | <i>Saccharomyces boulardii</i> |
| <i>Lactobacillus helveticus</i> | | <i>Saccharomyces cerevisiae</i> |
| <i>Lactobacillus johnsonii</i> | | |
| <i>Lactobacillus lactus</i> | | |

1.1. Fruit & vegetable based probiotics developments

Some researchers reported that fruit and vegetable based beverages may be the next category of food matrix to serve as carriers of probiotic bacteria [29]. Fruit juices have been recognized as novel and appropriate medium for fortification with probiotic cultures [30, 31]. Not only fruit juices, vegetable beverages also serve a good medium for the growth of probiotics [32]. Fruits and vegetables in both raw and fermented form provide a substrate for probiotic bacteria. Because the nutrients contained in the foods can easily be associated with probiotics [33]. However, lactic acid bacteria have special requirements and nutrients (essential amino acids and vitamins) for their growth. Scientists have found that there are some probiotic bacteria that can grow on fruit products and their growth ability depends on strains, features of substrate, oxygen content and acidity in the final product [34]. *Lactobacillus acidophilus*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Bifidobacterium lactis* are the most utilized probiotic bacteria for formulating new vegetable based probiotic products [35].

The general procedure of producing fermented and non-fermented probiotic enriched fruits and vegetable juices includes washing the relevant fruit and vegetable source, peeling and extracting the juice, filtration, filling into sterilized containers, post-sterilization, incorporation with probiotics and storage under refrigeration conditions. Additionally, fermentation is done after completing all the above steps for fermented product category and stored under refrigeration temperature [36]. However, the production process of probiotics based fruits and vegetables may vary based on the product type (such as salted, unsalted and mixed form); however, selection of suitable fruits and vegetables, cleaning, washing, disinfecting, blanching, peeling and shredding steps are common to all three types of products. The production of salted probiotic product with 2.5-10% salt involves soaking in a brine solution, fermenting for 5-30 days at 25-30 °C, drying or pressing, pasteurization, packing and storing respectively after completing common steps. The unsalted fermented fruit or vegetable products involve shredding, sun-drying, filling into vessels, fermenting for 1-2 weeks at 2-10 °C, pasteurization, packing and storing steps accordingly. Further, soaking in a brine solution, fermenting 1-2 weeks at 10-25 °C, drying, pressing, pasteurization, packing and storing steps are followed respectively after shredding for production of mixed products with 3-5% salt along with other ingredients [37].

Large red beet provides a raw material for production of probiotic beet juice by lactic acid fermentation with *Lactobacillus acidophilus*, *Lactobacillus plantarum*. These bacteria have an ability to produce greater amounts of lactic acid compared to other cultures. However, most of the culture viabilities in fermented beet juice are gradually lost during cold conditions while remaining *Lactobacillus plantarum* at 10^6 - 10^8 CFU/ml

after 4 weeks in 4 °C cold storage [38]. Grape juice also serve a matrix to deliver probiotics without addition of peribiotics (like insulin). Probiotic grape juice has developed through inoculating the *Lactobacillus paracasei* in to pasteurized ripen grape juice along with addition of proteolytic enzymes during juice processing to reduce the pectin content and for extraction of pigment and flavor compounds [39]. The probiotic carrot juice produced by incorporating *Lactobacillus acidophilus*, *Lactobacillus plantarum*, *Lactobacillus casei* and *Bfidum longum* at optimum conditions and do not require to provide any additional nutrient supplementation for retention of the viability of probiotic strains. Proximate probiotic carrot juice showed reduction in carbohydrate and increase in protein level than fresh carrot juice [40]. Pomegranate juice is fermented with incorporating *Lactobacillus plantarum*, *Lactobacillus delbrueckii* and they maintain optimal probiotic growth and viability during first two weeks of storage at 4 °C whereas *Lactobacillus acidophilus* and *Lactobacillus casei* lost their viability. Also scientist has found that citric acid which is the main organic acid present in the pomegranate juice is readily consumed by all probiotic microorganisms and indicate that the pomegranate juice may be a potential substrate for production of non-dairy based probiotic product [41].

Sauerkraut is made by using cabbage and salt and it is also consider as a probiotic super food due to spontaneous probiotic bacteria such as *Lactobacillus sakei*, *Lactobacillus plantarum*, *Lactobacillus hokkaidonensis*, *Lactobacillus rhamnosus*, *Leuconostoc spp.* This lactic acid bacteria provide enhance immune system function and help to prevent various illnesses while promoting the lactose digestion [42]. However, fermented cabbages did not show a significant change of sensory properties such as flavor [30]. Some researchers developed the probiotic based fermented yam with *Lactobacillus acidophilus* and it has found that the fermented yam could be produced without change in the allantoin and diosgenin content and thereby could provide many health benefits such as prevention of ulcers and inflammation [43]. Fermented tomato juice is prepared by *Lactobacillus acidophilus* and improve the sensory qualities of the final product [44]. Tomato juice provide a substrate for lactic acid bacteria except *Lactobacillus acidophilus*, *Lactobacillus plantarum* and *Lactobacillus casei* [45]. However, the probiotic bacteria has a capability of rapidly utilizing tomato juice for cell synthesis and lactic acid production without doing any pH adjustment [46]. Fermented banana puree is spongy soft solid, floating in a clear liquor which has been made by incorporating probiotics bacteria such as *Lactobacillus bulgaricus*, *Leuconostoc mesenteroides* [47]. Banana flour can be used for producing non-dairy yogurts, drinks and confectioneries with *Enterococcus durans*, *Enterococcus faecium* and *Lactobacillus curieae* like lactic acid bacteria strains [48]. Kimchi is a tasty traditional Korean fermented vegetables product which has been produced by incorporating group of probiotic lactic acid bacteria, especially, with *Leuconostoc mesenteroides*, *Lactobacillus plantarum* and variety of *Leuconostoc* and *Lactobacillus* species including *Leuconostoc citreum*, *Leuconostoc gasicomitatum*, *Leuconostoc gelidum*, *Lactobacillus brevis*, *Lactobacillus sakei*. It is a healthy food same as yogurt due to providing various good health effects such as cholesterol reduction, fibrolytic effect, anti-oxidative properties, anti-aging properties, brain facture, anti-mutagenic effect, anti-cancer effect, anti-obesity, anti-atherosclerotic and immunity potentiating functions. Cruciferous vegetables such as cabbage etc. are the main ingredient of Kimchi and sub ingredients include red pepper powder, garlic, ginger, green onion and contain various phytochemicals [49].

Moreover, some studies produced fresh cut apple slice wedges enriched with probiotic microorganisms by dipping apple wedges in an edible buffer solution namely probiotic culture to impart good physicochemical and sensory characteristics [50]. The probiotic peach jam has developed with *Lactobacillus rhamnosus*

strain and no any significant color modification was noticed in fresh produce form. Also it showed that peach jam might be a good candidate for producing novel non-dairy probiotic products with *Lactobacillus rhamnosus* strain either at refrigeration condition or at room temperature. Not only that the results have showed that daily intake of about 10 g of peach jam contribute to 10^8 - 10^9 viable probiotic cells with 45 and 78 days storage time period in room temperature and refrigerated conditions respectively. Contrast to the milk based probiotic products, those contain more than 10^6 probiotic bacteria per mL at the end of their shelf life which is normally around 30 days at refrigerated conditions [51]. Peanut cheese spread analog has produced by mixing peanut and water in 1:6 ratio with *Lactobacillus rhamnosus* and improved the functionality of product. However, the viability of probiotic strain is decreased from 2.34×10^{10} to 2.78×10^6 during 15 days storage period [52]. Noni juice which is a popular beverage originating in tropics has converted in to probiotic Noni juice by incorporating with probiotic bacteria such as *Lactobacillus plantarum*, *Bifidobacterium longum*. Because, probiotic bacteria has an ability to utilize Noni juice in cell synthesis and lactic acid production without external nutrient supplementation [53,54]. Almond milk is also a desirable matrix to obtained fermented derivative products through combining *Lactobacillus reuteri* probiotic bacteria and *Streptococcus thermophilus*. The process of fermentation modify the inner structure of almond milk by converting it in to a weak gel that facilitate retention of water content of almond milk [55].

1.2. Cereal based probiotic products

Not only the fruit and vegetable sources, but also the cereals provide a good substrate for growth of probiotic strains [56]. Namely, cereal and cereal based products offer chances to include probiotics prebiotics and fiber in human diet [57]. The constituents of cereal such as water soluble and insoluble β -glucan and arabinoxylans, oligosaccharides, resistant starch etc. are utilize to grow probiotic microorganisms [58]. Lactic acid bacteria such as *Lactobacillus fermentum* and *Lactobacillus plantarum* are the most common species with spontaneous lactic acid fermentation of cereal products and in addition to them *Lactobacillus* species such as *Lactobacillus casei*, *Lactobacillus sakei*, *Lactobacillus acidophilus* and *Lactobacillus salivarius* are also involved for the fermentation of cereal based fermented products [59].

Cereal grains are considered as important source of dietary proteins, carbohydrates, vitamins, minerals and fiber. However, nutritional quality of cereals and sensorial properties of their products may be poor compare to the milk and milk products due to some facts such as lower protein content, deficiency of certain essential amino acids (like lysine), lower availability of starch, presence of anti-nutrients like phytic acid, tannins and polyphenols and the coarse nature of the grain. Therefore various methods have been employed to ameliorate the nutritional qualities of cereals including genetic modifications and improvements, amino acid supplementation with protein concentrates or other protein rich sources, following the cooking, sprouting, milling and fermentation like processing technologies although probably fermentation is the best method [60]. In general natural cereal fermentation lead to decrease carbohydrate, non-digestible poly and oligosaccharides, improve the synthesis of certain amino acids and B vitamin availability, provide optimum pH condition for enzymatic degradation of phytate which contain in cereal in the complexes form with polyvalent cations including iron, zinc, calcium, magnesium, protein to increase the amount of soluble iron, zinc and calcium several folds [60–65]. Cereal products often ferment spontaneously, provide improved shelf life as well as better nutritional properties compared to the raw cereals [60]. The organoleptic properties of cereal based foods and dairy fermented foods which are

produced by lactic acid fermentation and are highly depend on organic acid amount in the product [66].

According to the probiotic growth studies on cereal based substitutes, malt medium encourage growth of probiotic population due to availability of maltose, sucrose, glucose, fructose and free amino nitrogen. Having sugar rich medium facilitate high chance of consumption of sugars during their exponential phase and result in lactic acid and acetic acid accumulation which lead to decrease the pH the medium. However, in contrast to malt, barley and wheat substrates provide lower cell population especially for *Lactobacillus acidophilus* & *Lactobacillus reuteri* due to availability of lower sugar content and lower free amino nitrogen concentration [66]. However, wheat and barley extracts show a significant protective effect on some probiotic microorganism's viability such as *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus reuteri* under acidify conditions [56].

The snack called 'Yosa' is a new product made from oat bran pudding cooked in water and fermented with both lactic acid bacteria and Bifidobacteria. The finish product texture and flavor of 'Yosa' is more similar to the yoghurt although it totally free from dairy or other animal product [67]. Therefore, it is a healthy addition to the diet due to contain of fiber and probiotic lactic acid bacteria. Further it is free from lactose, low in fat and β -glucan. Some researchers have found that the probiotic cultures such as *Lactobacillus reuteri*, *Lactobacillus acidophilus* and *Bifidobacterium bifidum* have a better ability to grown on non-dairy oats based products [67]. Oats consist with high soluble and insoluble fiber and it support for the fermentation ability of probiotic lactic acid bacteria. The probiotic oat drink has developed by incorporating *Lactobacillus plantarum* in to 5.5% oats, 1.25% sugar & 5% inoculum to obtain 10.4 log CFU/ml growth. The shelf life of the fermented oats drink extended over a period of 24 days and β -glucan level remained unchanged throughout both fermentation process and entire storage period [68]. Further, by using combination of barley slurry, sorghum slurry, garden cress seed powder, pumpkin seed powder with lactic acid bacteria starter culture containing *Lactobacillus acidophilus* and *Bifidobacterium bifidum*, a cereal based probiotic beverage has been developed and it showd 9 days shelf life under refrigeration storage at 4°C [69]. Moreover, the probiotic rice pudding is a better source of delivering probiotic by high levels of *Bifidobacterium lactis* with appreciated organoleptic quality and survivability of probiotics microorganisms has found in rice pudding for 14 days [70].

Soy bean based probiotic products

Soy bean and soybean derivatives show a good potential for functional food applications due to large amount of health beneficial components such as protein, isoflavones, fiber, essential fatty acids, oligosaccharide [71]. Many research studies have proven that soy is better medium for probiotic bacteria. Fermentation eliminate the soy taste namely beanie taste that is unacceptable for many consumers [72]. Production of soy milk by incorporating probiotic bacteria such as lactic acid bacteria increase its health benefits and also considered as a healthier than pure milk [73]. Use of Bifidobacteria for fermentation of soy is made protein more digestible, reduction of soy oligosaccharide, stacchiose and raffinose which are responsible to digestive problems [74,75].

Probiotic soy milk which is fermented through inoculating probiotic cultures such as *Lactobacillus acidophilus* and *Bifidobacterium spp* reflect a porcelain shine light color appearance, smooth texture and consistency without any characteristic odor [76]. Probiotic soy curd, formulated by incorporating enterocin producing culture *Enterococci faecium* and can be used as a therapeutic agent for food borne diseases

treatment such as Traveler's diarrhea, gastroenteritis [77]. Sogurt namely soy yoghurt has prepared from soy milk derived from germinated soy bean fermented by combining probiotics of *Lactobacillus helveticus*. The product has end up with better physiochemical, sensorial properties and improved texture properties (such increased adhesiveness, decreased hardness and gumminess) [77]. The probiotic soy cheese has made from soy milk fermented with soy cheese bacterial starter cultures combining with probiotic bacteria such as *Lactobacillus rhamnosus* and final sensory evaluation has found that those are not adversely affect on composition texture or sensory characteristics. The *Lactobacillus rhamnosus* can favorably utilize soy oligosaccharides as carbon source and they having capability of withstanding the technological processing of soy cheese [78]. Probiotic tofu is also a functional food which contains greater number of lactic acid bacteria and acceptable sensory characteristics that lead to enhance the consumer palatability. Acceptable sensory characteristics, high quality, high number of probiotic bacteria and appropriate stability are the features that shown by probiotic tofu [79]. Peanut-soy milk has produced by utilizing two important vegetable protein sources, peanut and soy which are readily available in abundance and at a reasonable cost and are comparable with protein content from animal based foods. For producing this beverage six different lactic acid bacteria have inoculated in to peanut-soy milk including probiotic lactic acid bacteria such as *Lactobacillus lactis*, *Lactobacillus rhamnosus* with other microorganisms [80]. Also in the way eligible probiotics and nutritionally improved functional soy product have yielded with the addition of soy fiber and probiotic kefir culture present better chemical composition and difference in color compared to the fermented products without fiber. Also, it has found that the functional soy product is with high firmness and also reduced syneresis compared to the fermented soy products without fiber and the product is considered as a better probiotic product due to retention of high *Lactococcus lactis* count during entire storage period [81].

1.3. Probiotic based bakery products

Bread is defined as a staple food in large segment of the world and an almost consumed every day. Also, bread is considered as a nutritious food product due to its composition including minerals, vitamins and dietary fibers [82]. However, application of probiotics in bread is challenging due to the fact that involvement of high temperature during baking process which could be significantly affected the probiotic cell viability [83,84]. Especially, temperature, moisture content, matrix structure like factors are affected to the retention of probiotic bacteria during baking through their affects on thermal and dehydration inactivation kinetics as far as consider the inactivation of probiotic bacteria in bread it can be seen that thermal inactivation is dominant compared to dehydration inactivation during baking [85]. Lactic acid bacteria such as *Lactobacillus brevis*, *Lactobacillus plantarum* have been frequently involved for the sourdough fermentation by giving acidification and proper structure to the grain and crubs. Also, some specific strains are involved to bread making who has ability to delay being firmness and staling [86]. Some studies has found that inoculation of *Lactobacillus plantarum* has an ability to produce dough similar as yeast fermented dough but with a larger dough volume [87]. Also sub strains of *Lactobacillus plantarum* and its active metabolites could be used to prolong the bacillus free shelf life of yeast leavened bread to 70 days at 30°C. Therefore it has recognized that bread is an interesting potential vehicle of viable probiotics [85]. *Bifidobacterium subtilis* is a spore forming bacteria who have an ability to sporulate when expose to harsh environmental conditions and its property confers major advantage when *Bifidobacterium subtilis* is used as a probiotic in food manufacturing processes such as baking that involve in temperature. When it is sproulated from *Bifidobacterium subtilis* has an ability to bear and survive heat shock, high acidity, high water activity and high sugar content. Also another advantage is it can be incorporate that microorganism in

any step in bread and bakery good during production process. It has found that probiotic *Bifidobacterium subtilis* strain remains stable under different processes such as baking, freezing, extrusion and that led toward excellent potential to use it as a candidate for incorporation probiotic in a different food product [88–90]. Incorporation of probiotics in to bakery product has shown improvement of several technological parameters including volume, specific volume, texture along with sensorial parameters such as flavor and aroma. Because of the harsh thermal stress happen during baking sourdough, technology along with probiotic micro capsulation has been studied as an alternative to enhance its beneficial effect such as nutritional value, increase cell viabilities though in few occasions [91].

1.4. Confectioneries based probiotic products

Confectionery is a type of often popular product obviously among children and youth allows to enrich diet with probiotic products which is given similar taste of traditional products. Refrigeration temperatures are not needed to store these products and hence being always “within reach”. Lactic acid bacteria viability in some products are high caused by low moisture content in product, keep of required water activity, below 0.6, high carbohydrate concentration mainly saccharose and limited access of oxygen. However the probiotics viability in confectioneries is basically depend on product recipe mainly the fat type, technological processes used for obtaining product processes time and conditions of storage. In fresh biscuits an increase of hardness is caused by properly tempered couverture, with properly crystallized fat in is V polymorphic form. But higher hardness of biscuits coated with couverture supplemented with lactic acid bacteria show that it has a better textural properties because of lactic acid bacteria addition is not hinder couverture fat crystallization. Obtaining confectionary cores used for couverture coating are done by mixing wheat flour, sugar, eggs and confectionary fat and raising agent in a ratio 100:30:20:10:1 [92].

1.5. Meat based probiotic products

The meat products such as raw cured and ripen meat have been traditionally produced through native or added carbohydrates fermentation process by lactic acid bacteria which are found in meat itself or in its environment. Manufacturing of probiotic meat products are relatively new to the industry and not well recognized field of meat industry [93]. Three main applications fields for use of such cultures can be seen in meat industry such as raw fermented sausages, raw cured hams and pasteurized, sliced pre-packaged meats (cold cuts) [94–98]. *Lactobacillus* strains such as *Lactobacillus sakei* and *Lactobacillus curvatus* are often predominant lactic acid bacteria which are used for raw fermented sausage production. In addition to them *Lactobacillus versmoldensis*, *Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus farciminis*, *Lactobacillus alimentarius* like *Lactobacillus* bacteria and *Leuconostoc* are also used for producing raw fermented sausages, but usually occur in significantly lower numbers [99]. Some present studies have indicated that there is a possibility to make dry sausages by incorporating probiotic or bioprotective organisms such as *Lactobacillus rhamnosus*, *Lactobacillus plantarum*. The number of lactic acid bacteria whether it is 8 log CFU/g or 9 log CFU/g in final dry sausage product does not affect either technological or sensory properties including flavour of produced dry sausages [100]. Also, it has found that the sensory and microbiological quality of fermented pork loin is depend on the used probiotic strain for fermentation process. *Lactobacillus acidophilus* has given the higher sensory quality after storage and the highest *Lactobacillus* bacteria count has given from *Lactobacillus casei*. Also it has indicate that addition of *Lactobacillus acidophilus* with 0.2% glucose in to pork loin show the lactic acid bacteria viability at the level 10^6 log CFU/g after 6 months storage [101].

2. Scope of future challenges

Keeping viability and stability of probiotic microorganism is a major technological and marketing challenge for industrial producers. Because both good viability and probiotic activity are considered as prerequisites for optimal functionality. However, some studies have shown that non-viable probiotics can have beneficial effect such as immune modulation and carcinogen binding in host [102,103]. Good sensory properties, phage resistance viability during processing, stability in the product and during storage like several technological aspects have to be considered when selecting the probiotics for processing [104]. Usually the commercially available probiotic cultures may consist with a single probiotic strain or mixture of several strains and most probably the probiotic properties are affected by the way in which strain or cultures has been produced [105]. Because of that specific adequate information on strain specific properties should be available for optimizing the process. Commercial interest is going toward the production of stable starter and probiotic lactic acid bacteria which contain large number of uninjured, viable cells and large savings of liquid and frozen concentrates are highly cost during transportation and storage. Culture stability improvements can be done using both freeze drying and spray drying preparations. Even though spray drying is more economical than freeze drying freeze drying is the most popular method to produce dried lactic acid bacteria preparations; because many lactic acid bacteria cannot tolerate high temperatures which are usually used during spray drying. Although freeze drying is less destructive to microorganisms when compared to the spray drying. Application of probiotic cultures on non-dairy based food matrix is a huge challenge. For probiotic containing baby foods, confectioneries like products it is very important that formulation maintains the probiotic viability and activity for extended period of time. Even though the probiotic cultures are included as ingredients to such kind of products usually multiplication is not happened which sets grate demands for the stability of probiotics. Water activity, oxygen tension and temperature like factors become increasingly significant when dealing with these kinds of products. Also, storing in room temperature is common for cereal products, drinks, confectioneries etc like non-dairy products and it can be led to overwhelming challenge for probiotic stability [104]. Use of probiotic encapsulation technology for ensuring probiotic stability sometimes overcome this problem [27].

Table 2: comparison of dairy and non-dairy based probiotics [106,107].

| Characteristics | Dairy based probiotics | Non-dairy based probiotics |
|----------------------------------|--------------------------------------|---|
| Products | Yogurts, fermented sour milk, cheese | Soy products, cereal based products, fruit and vegetable juices, fermented meat and fish products |
| Lactose intolerance | Adverse effect | No issue |
| Availability of calcium | Beneficial effect | No issue |
| High fat | Adverse effect | No issue |
| Cholesterol content | Adverse effect | No issue |
| Availability of dietary fibre | No issue | Beneficial effect |
| Digestibility | Not easy | Easy to digest |
| Probiotic survivability | High | Low |
| Flavour (diacetyl, acetaldehyde) | Beneficial impact | No issue |

| | | |
|-----------------|---------------------------------|---|
| Phyto chemicals | No issue | Adverse effect |
| Isoflavons | No issue | Beneficial effect |
| Product form | Most probably fermented in form | Most probably produced without fermentation |

Conclusion

Probiotic is a healthy microflora for human body. Due to rise of vegetarianism and consumer aware on alternative diet to dairy products, non-dairy probiotic products are developed. Several studies have investigated that probiotic can be incorporated with non-dairy based food matrixes including fruit and vegetables, cereals, soy products, confectioneries, meat products, bakery products. Consumption of foods along with probiotic effect promotes added health benefits to the human body. Incorporation of probiotic in to fruit and vegetables matrixes provide better sensory properties than other non-dairy based food matrices. Further, seaweed and legume sources are highly potential areas for incorporate probiotics which most people are not aware on.

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