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Formulation of Non-Dairy Probiotic Yoghurt in Context to Preclusion of Health Measures

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Abstract*: Increased consumer interest in health has driven a notable increase in the demand for functional foods, especially those containing probiotics. These beneficial microorganisms, recognized for their positive impact on health, are live bacteria or yeasts that offer advantages when consumed in appropriate quantities. They are commonly found in fermented dairy items such as yogurt but are now also making their way into non-dairy alternatives, addressing concerns related to dairy consumption.*

Yogurt, a widely acknowledged probiotic-rich food, is crafted through fermentation using specific strains of lactic acid bacteria. It provides various health benefits, including bolstering the immune system and enhancing the absorption of essential minerals and vitamins. However, the surging popularity of non-dairy yogurt options, derived from plant-based sources like soybean, almond, and coconut milk, illustrates a growing preference among consumers for lactose-free and cholesterol-free alternatives.

The criteria for producing and classifying probiotics and yogurt underscore safety, viability, and health advantages. Challenges in developing non-dairy probiotic products are also discussed, particularly in ensuring the viability of probiotics throughout processing and storage. Nevertheless, ongoing research endeavours seek to overcome these obstacles, aiming to optimize non-dairy probiotic offerings and provide consumers with a broader range of nutritious and functional food choices.

Keywords: probiotics

I. INTRODUCTION

Customer interest in health affects the nutritional habits and choices in the food chain. Consumers nowadays have been understanding about balanced nutritional diet and healthy food with more nutrient availability. There are some functional foods like probiotics containing, which come under the diet category of the nutritional food chain. Nowadays consumers have become more concerned and have started to learn about the proper diet maintaining food for their healthy life. The key to successful marketing and acceptance of new food depends on the concept of added value based on food quality, and food functions **(Rosi *et al.,* 2017).**

Recently, probiotic food marketing is grown very much because of the consumer awareness for their health. In today's world, probiotic products accounts for 60% to 70% of the total functional food market **(Hill C *et al.,* 2014)**.

Probiotics are the most common factor or increase in today's functional food as they are very beneficial if consumed in an appropriate amount **(Perricone M *et al.,* 2015)**. The microorganisms that are used as strains for making probiotics are health-beneficial, including intestinal and non-intestinal effects. Intestinal benefits include the prevention of diarrhoea, the reduction in symptoms associated with inflammatory bowel disease, the prevention of gastrointestinal cancers, the alleviation of lactose intolerance, and a reduction in *Helicobacter pylori* infections **(Markowiak P *et al.,* 2017)**. These probiotics can play an important role in the prevention and for treatment regarding intestinal inflammatory disorders such as Crohn's disease pouchitis, and paediatric atopic disorders. The impact of using probiotics on bacterial infections and immunological conditions such as adult asthma, cancer, diabetes, and arthritis is unconfirmed in humans **(Liu Y *et al.,* 2018)**.

There are dairy products which are having rich sources of probiotics present. Therefore, yoghurt is one of the products that has rich source of probiotics present. Yogurt, having functional ingredients are a type of functional food products, which are globally identified and have been accepted by consumers, where health and diet-conscious consumers considered yoghurt as a healthy product that helps in various ways to the body **(Liu L *et al*., 2022).** Globally, there are various types of yoghurts which are being produced with improved nutritional value, quality and healthier as well as on the basis of customers' requirements **(Ban Q *et al.,* 2022)** Yoghurt is the most consumable fermented milk globally by combining with pasteurized milk with lactic acid bacteria specifically Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus under controlled conditions **(Wijesekara A *et al.,* 2022)**.Consumption of the yoghurt can help to boost the immune system, aid in weight management, and also low downs the risk of cancer. In addition of more improved mineral and absorption of vitamins, respectively, because of the yoghurt, high protein constitutes, probiotics, lactic acid, bacteria, vitamins, calcium content and low-fat content **(Wijesekara A *et al.,* 2022)**.Therefore, the plain yoghurt where lack in various types of nutrients, such as phenolics, flavonoids, Anthrocyanins, irons and others **(Rashwan AK *et al.,* 2022)**.

Recently Non-dairy yoghurts are becoming more popular. These non-dairy yoghurts are Plant-based diet because of having much more nutrition value compared to dairy milk yoghurt. Non-dairy plant yoghurt products such as soybean, Mung Bean, Almond, Coconut, and Quinoa Milk have gained popularity among consumers and scientists due to some critical emergencies to consumers having lactose intolerance, high cholesterol, milk allergies, anaemia, and coronary heart diseases **(Huang K *et al.,* 2022).**

II. REVIEW OF LITERATURE

Probiotic Microorganisms

The term probiotic related to mankind since they started consuming fermented foods **(F. Guarner *et al.,* 1998)**. Probiotics is originated from the Greek language denotes for life. Probiotic is defined as living microorganisms, which after injection are good for health, but in specific numbers, which exert health benefits beyond inherent general nutritions (**Y. Rivera-Espinoza *et al.,* 2010).** The number of probiotics consumed by a normal healthy person should be 10^8 to 10^9 cells per gram.

The origin of the probiotic microorganisms had occurred from humans and animals and are present as normal flora in the gastrointestinal tract and mainly isolated from feces **(M.G. Gänzle *et al.,*2015).** However, there are many Microorganisms present that are isolated from fermented foods. Most of the probiotic microorganisms belong to lactic acid bacteria and Bifidobacterium. Lactic acid Bacteria are the most commonly used and as well as the important bacteria which is allied with human gastrointestinal. Lactic acid is the major end product for the sugar fermentation. The main LAB that are used as probiotics are from Lactobacillaceae family mainly *Lactobacillus acidophilus*, *Lactobacillus amylovorous*, *Lactobacillus casei*, *Lactobacillus crispatus*, *Lactobacillus delbrueckii*, *Lactobacillus gasseri*, *Lactobacillus johnsonii*, *Lactobacillus helveticus, Lactobacillus paracasei*, *Lactobacillus plantarum*, *actobacillus reuteri*, and *Lactobacillus rhamnosus* **(Mollakhalili *et al.,* 2020)** Other frequently used probiotic microorganisms are from the bifidobacteria family like *Bifidobacterium adolescentis*, *Bifidobacterium animalis*, *Bifidobacteriumbifidum*, *Bifidobacterium breve*, *Bifidobacterium infantis*, *Bifidobacterium lactis* and *Bifidobacterium longum* that mainly produce acetic acid and lactic acid. It is worthy to mention that some strains like *Enterococcus, Propionibacterium*, *Saccharomyces*, *Bacillus* and *Escherichia* genera have potential probiotic status. These genera are mostly given the generally-recognized-as-safe (GRAS) status, which indicates no or less health risks to the host upon consumption (**Ranadheera *et al.,*2010**). Probiotic bacteria present in the market is in the form of capsules but are mainly sold as fermented foods especially dairy products.

There are trillions of microorganisms which are present in a human gastrointestinal tract, consisting of about thousand or more variety of species and are also known as gut microbiota. The cut microbiota has an important role in organisms' health, because it helps to protect the immune systems and regulates the energy metabolism **(Marques *et al.,* 2014).** therefore, consumption of the probiotics has to maintain and enhance the intestinal microbiota. There are many studies which denotes that these probiotics helps in prevention and treating the diseases and also in health disorders, such as high blood pressure, serum cholesterol, lactose intolerance. **(Rasic *et al.,* 2003)** and many Gastrointestinal disorders like irritable bowel syndrome, crohn's disease, Peptic ulcers, antibiotic associated diarrhoea **(Quigley *et al.,* 2011).**

Probiotics start their effectiveness by one or more actions for example, creating a kind of restriction environment for the pathogenic microbes. The effectiveness of the probiotics can be achieved by lower down the pH values with the production of organic acids such as lactate, short chain volatile fatty acids, and also due to breakdown of complex carbohydrates **(LeBlanc *et al.,* 2017)** or elaboration of antibiotic-like substances such as bacteriocin like compounds. There are some of the bacteria like lactic acid bacteria which has the ability to adhere the intestinal epithelium and prevents the invasion of many pathogenic bacteria such as Escherichia coli, Salmonella and Clostridium spp which can be present in the gut epithelium and this phenomenon is known as competitive exclusion. Consumption of the probiotics can help to modulate both cellular and humeral immune system which enhance the host to resist against the pathogenic microbes **(Vidanarachchi *et al.,* 2007)**.

Criteria for probiotic production

As outlined by (**Gupta and Jeevaratnam *et al.,* 2018)** for a probiotic to be considered suitable for its intended purpose it must meet the following criteria.

- The probiotic should be deemed safe for human consumption, demonstrating, non-pathogenic and non-toxic characteristics.
- Probiotic must exhibit a high survival rate, showcasing resilience to bile toxicity and gastric juices.
- Possession of notable adhesion properties and the capability to effectively colonize Both the gastrointestinal and urinary tracks are essential requirements for probiotics.
- The probiotic should demonstrate stability, maintaining viability, over an extended period, both during storage, and throughout the product, shall life, as well as during the various stages of food fermentation processes.

Yoghurt

Globally, yoghurt has become one of the most popular fermented dairy products due to his nutritional and health benefits accordingly. Yoghurt has been originated back in the 6000 BC. The historical records of yoghurt back to 5000 BC, with its discovery attributed to nomadic communities, residing in the middle East, who extensively incorporated it into their diets, (**Rul *et al.,* 2017).** As an ancient, fermented food, yoghurt is known by various names globally, such as Dahi in India, Laban in Iraq and Lebanon, Zabadi in Egypt, and matsoni in Georgia, Russia, and Japan**.** The term yoghurt is derived from the Turkish word Yogurmak signifying the process of thickening coagulation or curdling. Additionally, yoghurt is a low-calorie food, providing approximately 90 Kcal per serving, meeting the recommended daily allowance for humans **(Rul *et al.,* 2017)**. Basically, yoghurt is a nutrient dense, fermented dairy product and is consumed by consumers due to its health benefits and for its nutritional use (**(Weerathilake *et al*.,2014).** Yoghurt is produced by using a starter culture of lactic acid bacteria strains, streptococcus thermophilus **(Xu *et al.*,2014)**. The US food and drug administration updated a description which is legal for the yoghurt and its basically for low-fat yoghurt and Non-fat yoghurt as evidence in the US code of Federal regulation in 21CFR131.200 **(code of federal regulation, 2022)**.

Yoghurt is recognized as a probiotic food item capable of delivering substantial quantities of beneficial bacteria that, upon ingestion, support the gut microbiome, thereby offering specific health advantages **(Weerathilake *et al.,* 2014)**. In the realm of food fermentation processes, microbial food cultures, particularly lactic acid bacteria (LAB), play a crucial role, with LAB being the predominant group of probiotics utilised in fermented dairy products. Another aspect of microbial cultures in food involves their functionality, tied to the capacity of live microbes to bestow health benefits upon consumers, categorizing these microbes as probiotic microorganisms. As per the FAO/WHO (2002) definition, probiotics are characterized as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host." Generally, certain probiotic microorganisms contribute to technological processes, while others are employed to enhance health attributes in the food product. Nevertheless, some of these probiotic microorganisms can independently fulfill both roles, and a single packet containing a combination of microbes can achieve dual objectives **(Mani-Lopez *et al.,* 2014).**

Criteria for yoghurt Classification

As defined by the FAO/WHO in 1984, yogurt is a coagulated milk product resulting from lactic acid fermentation catalyzed by Lactobacillus delbrueckii ssp. bulgaricus (Lb. bulgaricus) and Streptococcus thermophilus from milk and its derivatives. The microorganisms in the final product must be viable and abundant. If other bacteria, such as probiotics (e.g., Bifidobacteria, Lactobacilli spp.), are introduced, the product should be labeled as 'fermented milk' and cannot be termed yogurt. Furthermore, the Codex Alimentarius for fermented milk **(Codex STAN 24-2003)** specifies that yogurt should contain a minimum of 2.7% (m/m) milk proteins, a maximum of 15% milk fat, a minimum of 0.6% titratable acidity (expressed as a percentage of lactic acid), and a minimum of 10^7 colony-forming units (CFU)/g of microorganisms in the starter culture.

Dairy Based Products

A probiotic beverage made with diary involves enriching milk **(Benton *et al.,* 2006),** typically sourced from cows, but also potentially from goats, ship or water buffalo with probiotics **(Virtual Medical Centre 2020).** The standard production process includes pasteurisation, where the milk is heated to 71.7°C for 15 to 25 seconds, swiftly cooled, below 3°C to enhance self-life, and then aseptically inoculated with probiotic strains for fermentation. This method aims to deactivate spillage, micro, isms and enzymes, preserving nutritional contents. Conversely, non-probiotic drains, utilise substitute, fruits, vegetables and oatmeal **(Chaudhary *et al.,* 2019)**. For non-substrates sterilisation through autoclaving at 1:201°C at 15 psi for 15 minutes is necessary before fermentation **(Gangwar *et al.,* 2018)**

Since around 2010, coconut milk has gained popularity as a dairy substitute. It provides a range of nutrients, including proteins, lipids, carbs, and potassium, in addition to therapeutic advantages including antioxidant capabilities **(Daramola et al., 2016)**. Coconut milk has an average of 70 kcal per 100 mL, compared to 150 kcal per 100 mL in cow milk **(Katz, 2018)**. In addition to providing around 4% of the daily required amount of calcium, it is enriched with vital vitamins and minerals such as iron, phosphorus, B1, B3, B5, and C. Although 17% of these fats are saturated, lauric acid makes up around 87% of these fats, along with caprylic and capric acids (13%). Mother's milk contains lauric acid, which is mostly found in coconut milk and has been shown to be beneficial forOsteological health and brain development **(D'Amato et al., 2012; Paul et al., 2020).**

Milk contains minerals that are vital to human health and development, as well as to dairy-related activities including cheese-making and protein-salt interactions**(Franzoi et al.,2017).** Dairy products are a great source of calcium, protein, vitamin D, potassium, and phosphorus—all of which are essential for supporting good bone health**(Rizzoli et al.,2014)**

Allergens

In general, two primary milk proteins, namely casein and whey, are responsible for triggering allergic responses in humans. The acidification process of cow's milk results in two distinct fractions: Fraction 1, presenting as a solid coagulum known as casein, constitutes 80% of the total milk protein, while Fraction 2 appears as a liquid consistency called whey, contributing 20% to the overall milk protein content **(Arnberg *et al.,* 2012).** Allergenic components within the casein fraction include 32% aS1-casein, 28% B-casein, 10% aS2-casein, and 10% K-casein **(Schulmeister *et al.,* 2009)**. On the other hand, the whey fraction contains allergens such as a-lactalbumin (5%) and B-lactoglobulin (10%) **(Wal *et al.,* 2004)** along with traces of immunoglobulins, bovine serum albumin, and lactoferrin **(Restani *et al.,* 2009).**

Because allergens can exist in tiny concentrations and because different food matrices contain a wide variety of substances, it can be difficult to identify them in varied meal compositions. This is accomplished by employing a variety of analytical techniques, such as mass spectrometry, peptidomics, proteomics, genetic, and immunological approaches. Biosensors—devices that can recognize different compounds, such as toxins in the environment—also play a big part in this effort**(Hassani, S. et al.,2020, Huang, N. et al.,2015)**the vitamins**(Chauhan, D et al.,2019)**pesticide residue**(Li, G et al.,2020, Bucur, B et al.,2018)**and bio molecules in biological**(Hassani, S. et al.,2020)** or food samples**(Sun. X et al.,2015)**

Cholesterol content in dairy products.

Consuming a diet rich in fat and cholesterol significantly elevates the risk of developing cardiovascular diseases, a leading cause of global mortality, accounting for approximately 17.9 million deaths annually, as reported by the World Health Organization (**World Health Organization. Cardiovascular Diseases. 2021).** The intricate composition of dairy fat encompasses around 400 types of fatty acid species, with saturated fatty acids (SFA) constituting 65-70% **(Bard *et al.,* 2019)**. Research by (**Faye *et al.,* 2015)** revealed that fresh cow's milk has higher cholesterol levels (8.51 mg/100 g) compared to camel's milk (5.64 mg/100 g), coupled with a greater fat content in cow's milk (4.52 g/100 g) compared to camel's milk (2.69 g/100 g).

It is noteworthy that cholesterol is exclusively synthesized in animals, including humans, implying that animal and dairy-based products serve as the primary sources of cholesterol. In contrast, plant-based foods such as fruits, vegetables, nuts, and grains are devoid of cholesterol, as highlighted by Heart UK **(Afonso *et al.*, 2018).** The University of California San Francisco (UCSF) Health recommends a daily cholesterol intake not exceeding 300 mg.

Lactose intolerance

Dairy probiotic products are derived from bovine milk, which contains lactose, a sugar component found in animal-based milk. The breakdown of this sugar in milk requires lactase enzymes, specifically beta-galactosidase. The absence of these enzymes leads to an inability to hydrolyze or break down lactose into its monosaccharide units, namely galactose and glucose. When undigested or non-hydrolyzed lactose reaches the large intestines, bacterial enzymes degrade it, resulting in osmotic diarrhea, gastrointestinal pain, and flatulence for individuals who are lactose intolerant **(Khan *et al.*, 2021)**.

When the small intestine is unable to produce enough lactase, the enzyme needed to break down lactose, the sugar found in milk, lactose intolerance develops (**Lactose intolerance—symptoms and causes—Mayo Clinic).** The nomenclature used to characterize lactose metabolism is quite ambiguous, which frequently leaves people perplexed**(Misselwitz B et al., 2019).** When lactose is not absorbed and stays undigested in the gut, it can cause lactose malabsorption, which can result in bacterial fermentation in the intestinal lumen. After consuming lactose, this fermentation increases the osmotic load and causes intolerance symptoms**(Storhaug CL et al., 2017 , Forsgård RA et al.,2019).** Approximately 70% of adult humans worldwide have decreased lactase enzyme levels, with considerable regional and national variations**(Forsgård RA et al.,2019, EFSA Panel on Dietetic Products 2010, BaylessTM et al.,2017).** This condition results from the presence of other gastrointestinal illnesses or from lactase non-persistence caused by genetics. Lactose malabsorption (LM) and lactose intolerance (LI) might arise from either of the two situations**(Swallow DM et al.,2003, Misselwitz B et al.,2013).**People who have lactose intolerance (LI) are no longer recommended to abstain from all dairy products. Up to 5 grams of lactose per serving—roughly equal to 100 milliliters of milk—are tolerated by the majority of LI patients. When lactose is eaten with other nutrients, this tolerance level increases. It would be helpful in this regard to have a trustworthy resource for choosing items that don't exceed a person's lactose tolerance level**(Fassio F et al., 2018)**

Non-Dairy Probiotics

Fruits and vegetables are recognized as wholesome foods and serve as an excellent medium for functional ingredients due to their abundance in beneficial nutrients such as phytochemicals, antioxidants, minerals, vitamins, and dietary fibers**(Slavin*et al.,* 2012)**. Their elevated nutrient and sugar content play a crucial role in facilitating probiotic growth, and when combined with their rapid passage through the acidic conditions of the stomach, they contribute to high probiotic cell viability **(Kandylis *et al.,* 2016)**. Unlike dairy products, fruits and vegetables do not contain allergens, lactose, or cholesterol, making them more suitable for individuals with specific dietary concerns. Fruits, appreciated for their health benefits, refreshing nature, and appealing taste, have captured the interest of researchers in the development of probiotic beverages based on fruit juices **(Panghal *et al.,* 2018)**.

Probiotic bacteria ferment the carbohydrates present in fruits, vegetables, grains, and legumes to produce probiotic food, which causes the emission of alcohol and gasses**(Panghal et al., 2018).** Research has shown that the process of probiotifying fruit juice can result in tastes that are described as "bitter," "astringent," "dairy-like," "medicinal," "acidic," "salty," "dairy-like," "artificial," or "earthy" **(Luckow et al., 2004)** The sensory qualities of the probiotic juice might vary depending on the kind of fruit, the probiotic organism, the temperature at which they are stored, and the addition of prebiotics and protectants**(Lebaka et al., 2018).** Several investigations have demonstrated that probiotics have no effect on fruit juices' general acceptability. As an illustration, **(Perricone et al., 2015)**Adding 10% v/v of tropical fruit juices, such as passion fruit, pineapple, or mango, might help lessen the taste and smell of off-putting ingredients in non-dairy probiotic meals. Additionally, they showed that the flavor of pineapple juice containing Lactobacillus reuteri was unaffected. Masking is a potential solution to this problem, in which probiotics are mixed with pleasant scents and volatile substances to make them appear less present reported by **(Luckow et al., 2006).**

Challenges on non-dairy probiotics

The utilization of probiotic cultures in non-dairy products poses a significant challenge. As previously mentioned, the selection of the food matrix is crucial for ensuring the viability of probiotics throughout both the processing and storage stages. The survival and stability of probiotics in fruit, vegetable, and cereal probiotic foods are influenced not only by the food matrix, water activity, and pH of the final products but also by the specific probiotic strains chosen. Despite the presence of essential nutrients in fruit and vegetable juices, factors like low pH, attributed to high levels of organic acids and dissolved oxygen, may limit probiotic viability **(Shori *et al.,* 2016)**. Additionally, while dairy products are typically stored at temperatures around 5 °C, ensuring probiotic cell viability during the product's shelf life, the common practice of storing many types of non-dairy products at room temperature can pose a considerable challenge to probiotic viability **(Vinderola *et al.,* 2017).**

III. CONCLUSION

The rise in consumer interest in health has propelled the demand for functional foods, particularly those enriched with probiotics. These live microorganisms offer various health benefits when consumed in sufficient quantities, leading to their widespread inclusion in fermented dairy products like yogurt. However, the increasing popularity of non-dairy alternatives, stemming from concerns such as lactose intolerance and cholesterol intake, highlights a shift in consumer preferences towards healthier options. Yogurt, a well-known probiotic-rich food, boasts numerous health advantages, from strengthening the immune system to improving nutrient absorption. Despite this, the emergence of non-dairy yogurt options, made from plant-based sources, signals a growing demand for lactose-free and cholesterol-free alternatives. The production and classification criteria for probiotics and yogurt prioritise safety, viability, and health benefits, underscoring the importance of quality control in the manufacturing process. Challenges in developing non-dairy probiotic products, particularly in maintaining probiotic viability, present hurdles yet to be fully addressed. Nonetheless, ongoing research efforts are dedicated to overcoming these challenges, aiming to optimize non-dairy probiotic offerings and expand the range of nutritious and functional food choices available to consumers. As the market continues to evolve, it is crucial to prioritise both innovation and consumer health concerns, ensuring that functional foods remain a vital component of a balanced and healthy diet.

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