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Nutritional Benefits of Enriching Dairy Foods with Probiotics

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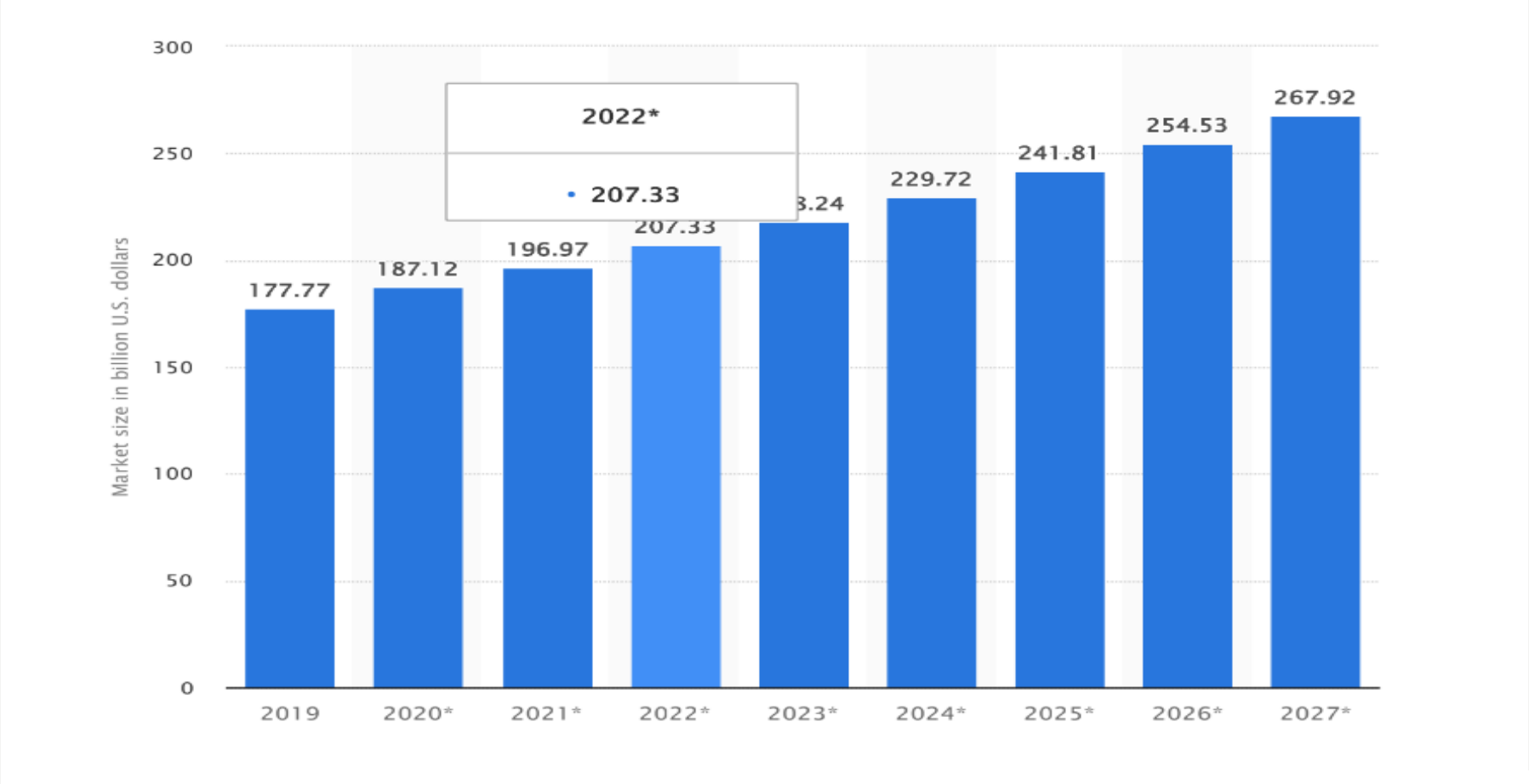
FOOD, FUNCTIONAL FOOD, DAIRY AND PROBIOTICS

- Food is essential for life
- Functional food is one step ahead that gives something more than proving basic nutrition
- Probiotic food products represent one of the important categories of functional foods
- Dairy provides the best matrix for carrying probiotics
- Several food products prepared with the help of beneficial microbes have been consumed by mankind since ancient times.
- Beneficial microbes are the agents that produce many fermented foods and beverages
- Indications about the use of products with healthy microbes are given in *Vedic* literature and are also depicted in ancient Egyptian and European treatises.
- Systematic studies on probiotics started after the publication of the book “Prolongation of life” by Eli Metchnikoff in 1907.

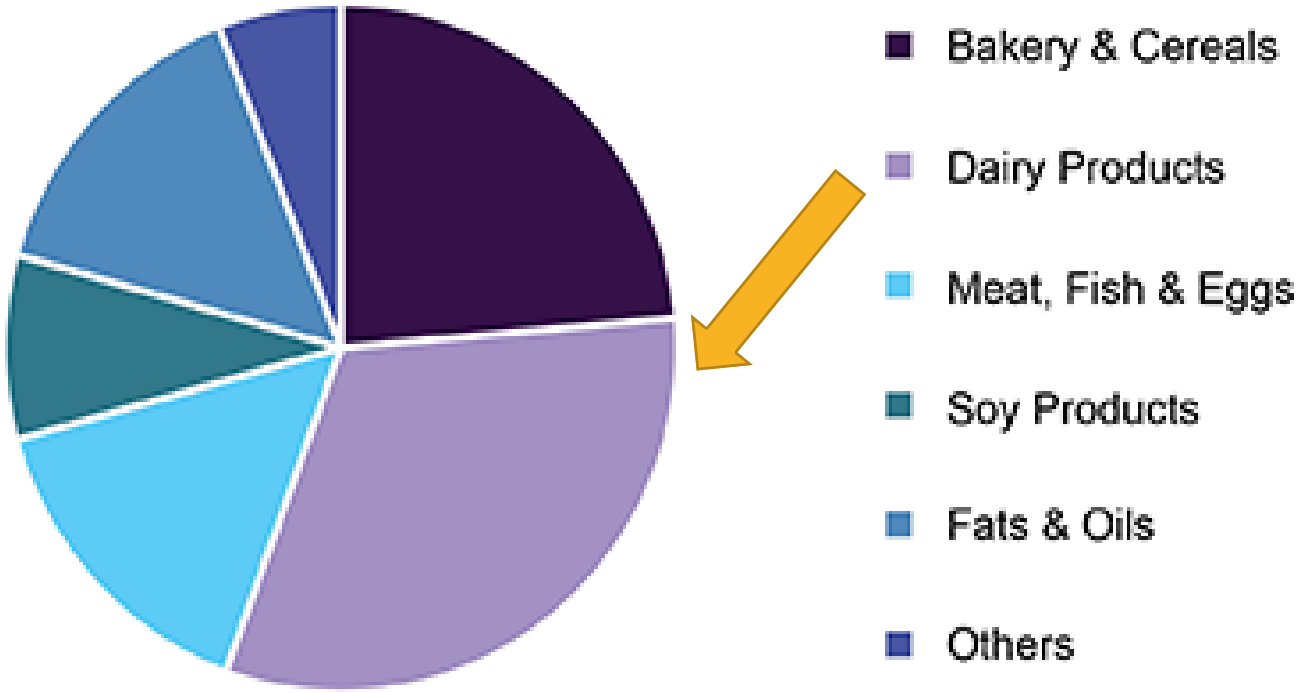
HOW BIG IS THE FUNCTIONAL FOOD MARKET WORLDWIDE? (BILLION USD)

267.92 billion USD in 2027



https://www.statista.com/statistics/1061728/india-functional-food-retail-market-value/

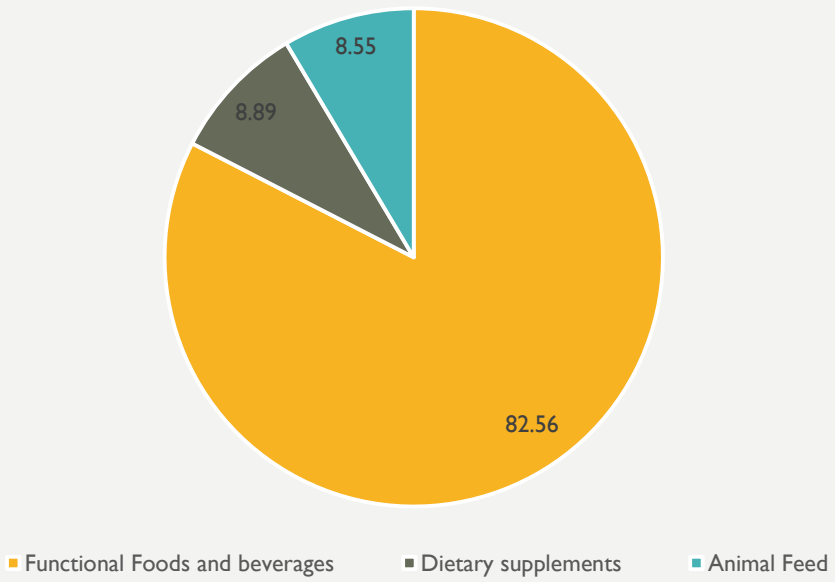
Global functional foods market share, by product, 2018 (%)



Source: www.grandviewresearch.com

World Probiotic Market

Market Share-2016



Probiotic Market – CAGR 2016-2021

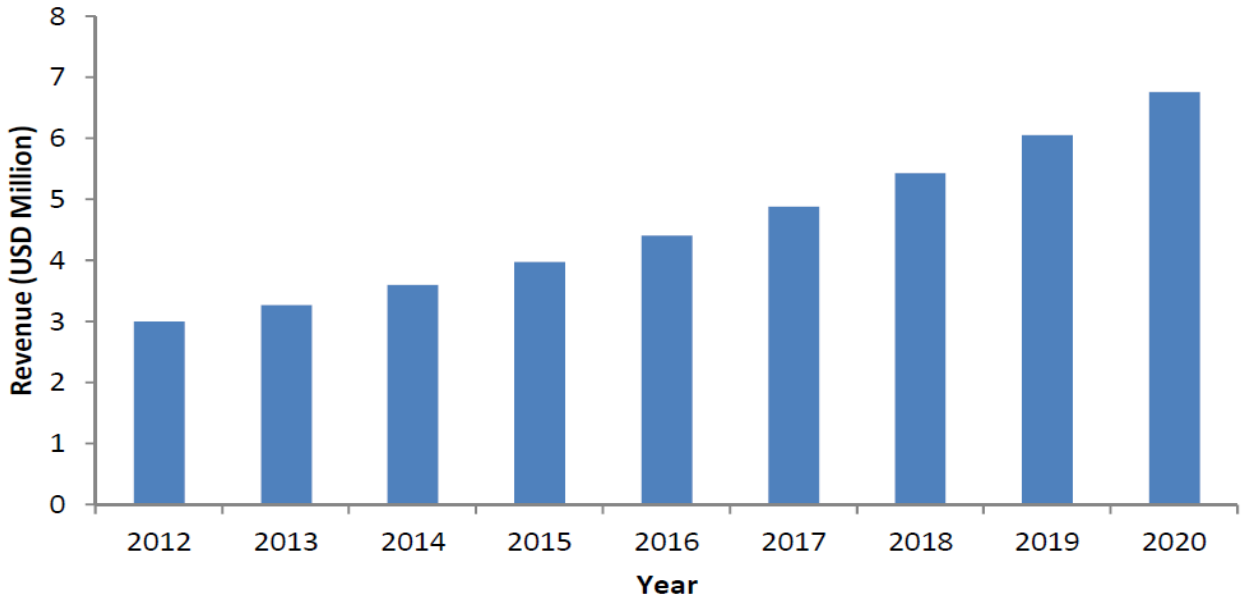
Functional Foods and beverages	**8.20%**
Dietary supplements	8.84%
Animal Feed	7.41%

A report by Global Market Insights Inc. indicates that probiotics market will be worth USD 4.15 billion by 2027 and growth is expected to register 9% Compound Annual Growth Rate (CAGR) between 2021 to 2027

INDIAN SCENARIO

- Indian functional food market is growing at 17%, while probiotic market is expected to grow at the rate of 20% per annum, doubling in next 5 years.
- The Indian market for food ingredient Probiotics is largely import driven.

Figure 30: Indian Probiotic Ingredients Market Revenue, 2012-2020



Source: Frost & Sullivan

Combination of Dairy Foods, Fermentation and Probiotics

- Consumption of milk and milk based products like yoghurt, dahi, cheese is age old and widespread.
- The art of fermentation of foods is also know since ancient times
- In Indian culture, product know as "*Panchamrut*" is know since Vedic times and is a must food in every ritual – it has fermented milk and honey as major ingredients that makes present day "Synbiotic"
- The process of fermentation has many advantages –
  - breaks down complex nutrients,
  - improve digestibility,
  - improve shelf-life,
  - improve organoleptic properties and
  - the process is simple, user friendly, environment friendly and safe.

Indigenous Fermented Dairy Products

Mallappa *et al.*, 2020

Why **Dairy based ?**

- Milk has been consumed by mankind since ages and is considered nature's almost complete food.
- Products developed with milk as the raw material does not need to prove their nutritional value.
- Milk has capacity to act as a natural buffer against the stomach acids thus protecting the bacteria.
- Dairy provides excellent matrices that protect probiotic bacteria

The combination of milk, probiotics and fermentation has yielded extraordinary nutritional and health benefits and hence about 65% of probiotic foods in the market are dairy based.

EFFECTS OF FERMENTATION ON MAJOR NUTRIENTS

Carbohydrates

Broken down by fermenting microbes to simple sugars, organic acids and flavour compounds

Fermented milk can be easily consumed by lactose intolerant people

EFFECTS OF FERMENTATION ON MAJOR NUTRIENTS

Proteins

The proteins become more easily digestible in fermented products because;

- ✓ the microbes pre-digest the complex compounds and produce simple peptides and amino acids which can be quickly absorbed by the body
- ✓ Natural fermentation of cereals increases net protein utilization and available lysine
- ✓ In fermented milks, the ingestion of protein in presence of lactic acid, results in softer curd formation

This is especially useful for people with gastric atrophy, gastro-intestinal disturbances and protein mal-nutrition.

EFFECTS OF FERMENTATION ON MAJOR NUTRIENTS

Lipids

Only slightly affected in most of the bacterial fermented foods like dahi, yoghurt, etc.

However, this little effect may give some volatile fatty acids contributing to flavour and some fatty acids which have therapeutic importance.

EFFECTS OF FERMENTATION ON MAJOR NUTRIENTS

Minerals

- Fermented milks are excellent source of calcium and phosphorus which are essential for bones.
- Absorption of calcium and other minerals is higher in fermented milks due to presence of lactic acid.
- Hydrolysis of chelating agents such as phytic acid during fermentation, improves the bioavailability of minerals.

EFFECTS OF FERMENTATION ON MAJOR NUTRIENTS

VITAMINS

Fermentations that increase the content or availability of vitamins such as thiamine, riboflavin, niacin or folic acid can have profound direct effects on the health.

Biological enrichment of foods via fermentation can prevent occurrence of several diseases

Probiotic Microorganisms

FSSAI List of Approved Probiotics as per Schedule VII
In force from 6th September 2021
FBOs to comply with the provisions by 1st April 2022

	Name of the Microorganism
1	*Lactobacillus acidophilus*
2	*Lactiplantibacillus plantarum*
3	*Limosilactobacillus reuteri*
4	*Lacticaseibacillus rhamnosus*
5	*Ligilactobacillus salivarius*
6	*Lacticaseibacillus casei*
7	*Levilactobacillus brevis*
8	*Lactobacillus johnsonii*
9	*Lactobacillus delbrueckii* sub- sp. *bulgaricus*
10	*Bacillus coagulans*
11	*Limosilactobacillus fermentum*
12	*Lactobacillus caucasicus*
13	*Lactobacillus helveticus*
14	*Lactobacillus lactis*
15	*Lactobacillus amylovorus*
16	*Lactobacillus gallinarum*
17	*Lactobacillus delbrueckii (Lactobacillus delbrueckii subsp. delbrueckii)*
18	*Bifidobacterium bifidum*
19	*Bifidobacterium lactis (Bifidobacterium animalis subsp. lactis)*
20	*Bifidobacterium breve*
21	*Bifidobacterium longum (Bifidobacterium longum subsp. longum)*
22	*Bifidobacterium animalis (Bifidobacterium animalis subsp. animalis)*
23	*Bifidobacterium infantis (Bifidobacterium longum subsp. infantis)*
24	*Streptococcus thermophilus (S. salivarius subsp. thermophilus)*
25	*Saccharomyces boulardii (Saccharomyces cerevisiae subsp. boulardii)*
26	*Saccharomyces cerevisiae (Saccharomyces cerevisiae subsp. cerevisiae)*
27	*Lacticaseibacillus paracasei*
28	*Lactobacillus gasseri*
29	*Bacillus clausii*
30	*Established probiotic strains of Bacillus subtilis*

New generation probiotics

- *Faecalibacterium prausnitzii*
- *Akkermansia muciniphila*
- *Bacteroides fragilis*
- *Bacteroides uniformis*
- *Eubacterium hallii*

Mode of Delivery of Probiotics

- Probiotics can be incorporated in food products and consumed in different ways.
- The method of incorporation depends upon the Type of Raw Material, Processing Conditions, Type of Cultures and their Biological Activities and Consumer Preferences.

Probiotic as ingredients

Probiotic as adjunct culture

Probiotic as starter

DISPENSING PROBIOTIC IN PRODUCT

- **Using probiotic organism as starter culture** - this type of products will have most active probiotic cells in maximum possible numbers and will give benefit of its metabolites produced in the product itself.
- **Using probiotic culture as supplementary flora** – probiotic is added as a culture along with a good starter culture. The product qualities are developed by the main starter culture, while probiotic cultures remains under low profile during manufacture.
- **Using probiotic culture as food ingredient** - probiotic is added as an ingredient at a specific stage during product manufacture. However, it should remain viable and reach the intestinal tract of the consumer and function there.

POSSIBLE PRODUCT FORMATS

- Natural set products like dahi
- Stirred product like lassi
- Beverage like butter milk
- Popular product like Raita
- Dried formulations for higher shelf-life

Products manufactured with probiotics as the sole culture

Probiotic culture(s) is added to the product and it may grow and bring out metabolic changes

- Acidophilus products
- Bifidus products
- Yakult
- Others

Acidophilus Products

- Acidophilus products are cultured Milk products made by fermentation with *Lactobacillus acidophilus* alone or in combination with some other Bacteria or Yeasts (Prajapati & Behare, 2018).
- This culture was one of the oldest candidate as Probiotic.
- Acidophilus Milk has a high biological value, due to the availability of essential Amino Acids of milk proteins and microbial cell protein.
- Folic Acid, Riboflavin, Thiamine, Niacin, Pyridoxine and Vitamin K, all of which are slowly absorbed by the body, have all reportedly been shown to be synthesized by *Lactobacillus acidophilus*.
- In milk cultured with *Lb.acidophilus*, several free amino acids, viz., lysine, histidine and arginine increased considerably as a result of fermentation. It showed 30% increase in thiamine and 10% increase in cobalamin and riboflavin contents (Alm, 1982)

- Acidophilus Milk did not become popular due to its unappetizing taste.
- Several other products which supply large numbers of viable Acidophilus cells, like cold pasteurized milk, ice-cream, aco-yoghurt became popular.

Bifidus Products

- Bifidiobacteria are normal inhabitants of the intestinal tract of newborns and infants.
- The Milk cultured with Bifidobacteria is called Bifidus Milk.
- Human strains of *Bifidobacterium bifidum* or *Bifidobacterium longum* are used as culture.
- It has been used to protect against gut microflora imbalances, in the treatment of chronic constipation, liver diseases, and also as an aid in the treatment of gastrointestinal disorders (Kurmann *et al.*, 1992) .
- In the Dairy Industry, Probiotic strains of Bifidobacterium are used in development of Humanized Milks or Baby Foods and they are also used as a component of the starter culture intended for the production of Fermented Beverages, Cottage Cheeses, Ice Creams, etc. (Hati S et al., 2013)
- Bifidus Milk supports the treatments of Gastrointestinal Disorders and Liver Diseases and is digested easily (Yerlikaya 2014).

Products manufactured with probiotics as the secondary culture

These products are made with regular lactic cultures, while the probiotic strain remains secondary. However, it is active and growing at a lower speed.

- Yoghurt
- Cheese
- Probiotic dahi
- Kefir
- Butter milk
- Lassi
- Kumiss

Yoghurt

- Yoghurt is a fermented milk product popular world over is made by fermentation of milk with symbiotic cultures of *Streptococcus thermophilus* and *Lactobacillus bulgaricus*
- It is know for its nutritional and therapeutic value, however none of these two cultures survive in GI tract.
- Probiotic Yogurts have become more popular, where selected strains of Lactobacilli and/or Bifidobacteria are added as supplementary flora [Kurman, 1984, Kurdal, 2014]
- The Bio-Yogurt supplemented with ascorbic acid was shown to improve the viability of *L. acidophilus* [Tamime *et al.*, 2005]. One of the possible mechanisms that may help to improve the viability of probiotic bacteria is the oxygen scavenging effect of ascorbic acid.
- The addition of whey proteins may enhance the viability of some probiotic bacteria as well, due to their buffering capacity, especially in yogurts with added fruit pulp.
- The incorporation of prebiotics, like fructooligosaccharides and inulin [Vinderola *et al.*, 2000], nutraceutical's containing isoflavones, phytosterols, and omega-3-fatty acids [Kailasapathy *et al.*, 2008] in yogurt formulations stimulated the viability and activity of probiotic bacteria.
- Also, the addition of cysteine to yogurt at 250 and 500 mg/L was proven to be associated with higher viability of *L. acidophilus* during manufacture and storage.
- Probiotics help in improving the digestibility of proteins, absorption of minerals and synthesize some vitamins.
- Helpful in control of lactose intolerance is the only approved health claim in Europe by consumption of yoghurt.

Probiotic Dahi

- Dahi is a popular fermented milk prepared in India with mesophilic lactic acid bacteria and know for its palpability and therapeutic effects since centuries.
- In most of the industrial processes, thermophilic cultures, which set the curd ins short time used.
- A Probiotic Dahi is made by addition of selected strains of probiotic *Lactobacilli* or *Bifidobactetria* as additional culture.
- Some initial studies on Probiotic Dahi include the preparation of Acidophilus Dahi at Sheth MC College of Dairy Science, Anand where the product had live Lactobacilli count of 200 millions/g and proved to implant in intestinal tract (Prajapati et al, 1986). Feeding of probiotic dahi resulted in significant reduction in the Serum Total and LDL Cholesterol.
- Work conducted at National Dairy Research Institute (NDRI) on Dahi with increased functionality. Probiotic dahi significantly enhanced the Non-Specific immune response by increasing Phagocytic activity as well as Lyposomal enzymes activity of Peritoneal Macrophages. It also effectively enhanced the protective immune response against *Salmonella enteritidis* [Yadav et al, 2007]
- Milk added with cereals/millets have also been fermented to have better nutrional value, especially presence of fibre. [Kale et al, 2011].

Probiotic Lassi and Butter milk

- True butter Milk is the fluid remaining after the Cream is churned into Butter. However, more commonly the cultured Butter Milk is produced by Souring Skim Milk with Mesophilic Lactococci and Leuconostocs .
- Lassi and butter milk are the liquid forms of yoghurt /dahi
- Probiotic lassi/butter milk contains added strain of Lactobacilli or Bifidobacteria, which improves some of the biological functions of the product.
- Probiotic butter milk is the cheapest but most nutritious and healthy product for the masses and hence should be promoted in mid-day meal schemes in schools.
- Lot of work has been done on Probiotic Lassi or *Chaach* or Butter Milk in India. Several such products are developed and tested at AAU, Anand [Senan et al, 2015].
- The Bioactive Peptide found in Lassi showed partially or completely homology to that the Milk Protein Bioactive peptides having ACE-Inhibitory, Immunomodulatory, Antioxidant, Opioid and Cytomodulatory Activities [Padghan et al., 2017].

Product	Ingredients	Remarks
Synbiotic Dahi	Milk, Inulin, Sugar	Set Coagulated Product with 10^8 Viable Cells of Probiotic Lactobacilli per gram.
Synbiotic Raita	Milk, Inulin, Fructooligosachharide, Tomato, Cucumber, Onion, Banana, Sapota, Sugar	Stirred Yoghurt Type Products Fermented by Probiotic Lactobacilli and Garnished with Fruits and Vegetables.
Synbiotic Lassi	Milk, Oat, FOS, Carrot, Mango, Sugar, Honey	Thick Liquid with Probiotics and Shelf Life of 3 weeks at 5°C.
Whey Drink	Whey, Sugar, Pineapple	Beverage with Fruit Pieces and 10^8 cells/ml of Probiotic Lactobacilli.
Herbal Probiotic Lassi	Milk, Safed Musli, Sugar, Honey	Milk Fermented by Probiotic Lactobacilli and Supplemented with Herbs.
Protein Rich Lassi	Milk, Spirulina, Sugar	Fermented Milk Enriched in Protein by Spirulina.
Acidophilus Banana Powder	Acidophilus Milk, Banana, Sugar, Elachi	Dried Product with 10 million/g Viable Cells of Lb. acidophilus.
Acidophilus Wheat Malt Powder	Acidophilus Milk, Wheat Malt, Sugar, Cocoa Powder	Dried Product with 10 million/g Viable Cells of Lb. acidophilus.
Milk-Rice Probiotic Food	milk, Rice, Freeze Dried Probiotic Culture	Milk and Rice were Fermented and Spray Dried and Blended with Freeze Dried Probiotic Lactobacillus Cells.
Probiotic Carbonated Beverage	milk, Sugar	Milk Fermented by Probiotic Lactobacilli and Supplemented with Artificial Carbonation
Carbonated Probiotic Beverage Employing Yeast	Paneer Whey, Sugar	Whey Fermented with L.helveticus and K.marxianus @ 2 % v/v)
Probiotic Ragi Ice Cream	Finger Millet, milk Solids	Ice-Cream Supplemented with Finger Millets (Ragi) and added with Indigenous Probiotic Culture
Probiotic Oat Based Lassi	Oat, Whey Protein Concentrate	An Oat based Fermented Beverage using Oat Bran, Cow Milk and Probiotic Bacteria
Probiotic Smoothie	SMP, WPI, Oat, Sugar, Mango Pulp	Smoothie was Prepared using Dry Dairy Ingredients

KEFIR

- Kefir is a foamy, effervescent Milk product resulting from mixed Lactic Acid and Alcoholic Fermentation of Milk by Kefir grains
- Kefir is expected to contain several potentially Probiotic organisms and it has been found to show health benefits.
- It improves digestion, prevents constipation and regularizes Bowel Movement.
- Being a natural antioxidant it helps to keep skin youthful and glowing. Kefir can enhance the functioning of Brain and aids in reducing stress.
- Regular use of Kefir helps to reduce high Cholesterol level. Similarly Kefir is found effective for Heart health, respiratory and immune system.
- Kefir has been associated with cholesterol metabolism, Angiotensin-Converting Enzyme (ACE) inhibition, increased speed of wound healing, tumor suppression, antimicrobial activity, and modulation of the immune system including the alleviation of allergy and asthma.

KOUMISS

- Koumiss is a product similar to Kefir made by Acid and Alcoholic Fermentation of Milk.
- Koumiss culture consists of *Lb. delbrueckii* subsp. *bulgaricus*, *Lb. acidophilus* and *Kluyveromyces lactis or K. marxianus* subsp *marxianus*
- In Russia, Koumiss is used for the treatment of Pulmonary Tuberculosis. The causative agent of Tuberculosis (*Mycobacterium tuberculosis*) cannot survive in the Mare Milk because of the Antituberculosis element generated by the microflora of Koumiss [Zha M, 1987].

Probiotic Cheese

- Cheese is one of the most versatile food products available today – more than 400 varieties are available
- Development of Probiotic Cheeses requires knowledge of all their processing steps, other microbes present and biochemical changes going on during long storages.
- Compared to Yogurt and Fermented Milks, cheese holds multiple advantages as a probiotic carrier.
- They have a higher pH and therefore buffering capacity [Karimini *et al.*, 2011], they have high energy, are particularly nutritious, have a higher fat content, and can survive a longer shelf life while maintaining higher viability, which has been demonstrated through multiple studies.
- Probiotics in Cheese were found to survive the passage through the simulated Human Gastrointestinal Tract and significantly increase the numbers of Probiotic cells in the gut.
- Cheese was produced by two methods using Cheese Starter Cultures (Probat 505) in combination with 0.04% of Probiotic *Lactobacillus fermentum* strain ME-3 (10^9 CFU/mL) with high Antimicrobial Activity and Antioxidative properties
- Encapsulated *B. infantis*, *B. bifidum*, and *Bifidobacterium longum* have been used in the manufacture of cheeses like Crescenza cheese, and may be used to improve viability and protect various Probiotic organisms [Kailasapathy , 2002].

Probiotics used as supplements

These are regular products added with the required dose of probiotic cells. The product matrix is supposed to provide support.

- Fermented dairy products
- Cheese
- Chocolates
- Nutrition bars
- Infant foods
- Ice creams
- Frozen yoghurt
- Smoothies

Fermented Dairy Products

- Usually freeze-dried cells of Lactobacilli and /or Bifidobacteria have been added as an ingredient at the various stages of manufacture of fermented milks.
- Microencapsulation is one of the important tools to improve the viability of Probiotic cells in different products.
- The best physical properties (Low Moisture, Hygroscopicity, and Solubility values with High Bulk and Tapped Densities), were found in microcapsules produced with Inulin and Gum Arabic .

Ice-Cream and Frozen Desserts

- Frozen Desserts such as ice cream have potential to be carriers of Probiotic organisms, but the freezing process could jeopardize culture viability during manufacturing and storage.
- Multiple combinations of Bifidobacteria and Lactobacilli have been used in ice cream at a dose of 1 to 100 million cells/ml [Tamime 2005].

Probiotic Chocolates

- Chocolate could offer a good alternative for Probiotic applications compared to common Dairy Products.
- Supplementation of Milk Chocolate with Inulin (5%) and free or Encapsulated Lactobacilli (~8 log CFU/ g) increased the Faecal Lactobacilli, and decreased coliforms and b-Glucuronidase activity [Mandal *et al.*, 2013]
- Probiotic Chocolate prepared using *Lactobacillus helveticus* MTCC 5463 had acceptable organoleptic quality till 30 days of storage, but the viability of probiotics (2.42×10^8) remained good only up to 15 days of storage at 10 ± 2˚C [Ghadiya et al, 2018].

Infant foods

- Attempts were made to modify the Cow's Milk composition to bring it closer to Human Milk.
- Bifidobacteria play a key defensive role in the baby's gut by modulating the gut flora.
- Some of the earlier products that came from Europe were prepared as a dried formula product containing Prebiotic Lactulose and viable *B. bifidum*.
- One of the most thoroughly studied Probiotic strains available is *B. animalis subsp. lactis* BB12, which has been commercially available for more than 25 years.
- Cesarean-delivered infants that consume formula supplemented with Bb12 have higher levels of Anti-Poliovirus Specific and Anti-Rotavirus Specific IgA.

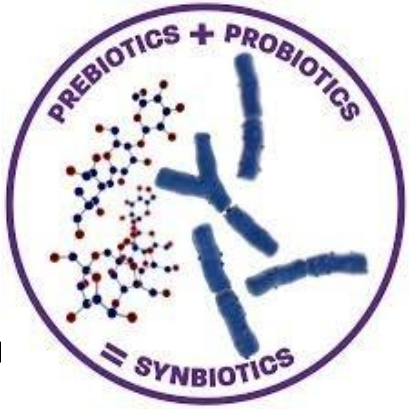
THE CONCEPT OF BLENDING…..

- Milk is one of the best substrate for making fermented foods, being nutritionally proven and rich medium for growth of lactic acid bacteria.
- However, milk solids are costly and are not affordable by millions of people in the developing world.
- Blending of cheaper cereal solids with milk during fermentation can reduce the cost of the product.
- Nutrients of milk and cereals give a complimentary effect and help reduce the deficiencies of each other.

Hence, several probiotic products with blending of milk with cereal, pulses, fruits, vegetables have been prepared, which showed;

- Higher proportion of essential amino acids
- Higher quantity of B- complex vitamins
- Presence of digestible fiber

Synbiotics as a means of nutritional enrichment



- Several prebiotic compounds have been incorporated in foods along with probiotics to impro viability and efficacy of the probiotic cells.
- Supplementing foods with Prebiotics, benefits to host nutrition, inhibit growth of pathogens, and promote beneficial microbiota [Delgado *et al.*, 2011] .
- Causes fermentation of nondigestible fibers, promote synthesis of B and K Vitamins, produce SCFA and polyamines, improve gastrointestinal motility and Function, reduce cholesterol and stimulate local immune system [Penders *et al.*, 2016].
- Increased expression of the binding proteins or active carriers associated with mineral absorption.
- Enhanced immunity and modulation of mucin production.

TO CONCLUDE……

- Probiotic foods are becoming increasingly popular in the society as they are being projected as food cum medicine.
- Milk has been an excellent raw material for Probiotics to grow and it is known for its Nutritional Value, being nearly a complete food.
- When probiotics are incorporated in dairy matrices, it shows positive effects on quantity and quality of nutrients in the foods -excellent quality proteins rich in essential Amino Acids, Vitamins, easily absorbable Minerals, Vital Fatty Acids and many more Micronutrients for the body.
- Application of Probiotics in prevention and supplementary treatment of several ailments is well known as evident by several clinical trials.

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We are grateful ……

Thank you all for patient listening

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