FERMENTATION ASSISTED FUNCTIONAL FOODS.

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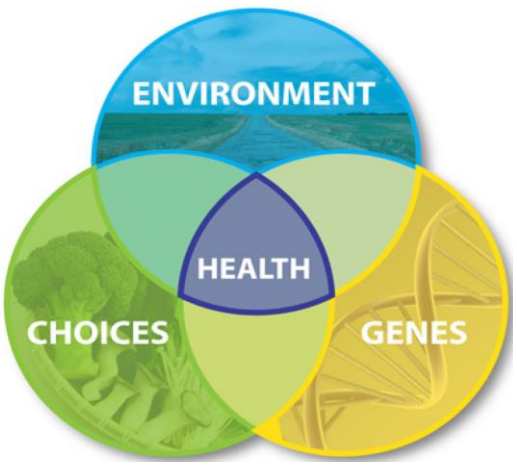
Introduction

Food is the utmost basic need of life that meets the nutritional demand of an individual. Fats, carbohydrates, proteins, vitamins and minerals are the nutrients needed for growth and maintenance. Dietary fiber, phytochemicals, antioxidants, probiotics, prebiotics, etc. are considered as non-nutrient factors that boost human health with their positive influence on the physiology of host and global epigenetic imprints. Nutraceuticals or functional foods or foodiceuticals or medifoods are the food categories that may act as prophylactics or therapeutics and whose dietary inclusion may regulate the disease controlling mechanisms .

Food particularly the traditional can optimize human health and environmental sustainability if provided in right quantity and quality. It is a great challenge to provide healthy diets using indigenous food processing techniques. Since time immemorial, fermented foods have been traditionally valued and are usually categorized as “functional foods” owing to their health benefits. Functional foods are reported to support basic nutrition, affirming good health and longevity with natural ingredients as influential drivers . Fermentation brings about desirable changes in food constituents by enhancing the palatability, organoleptic properties and bioavailability of nutrients and altering the functional properties of food and also facilitating the viability of beneficial probiotic bacteria. Fermented foods comprising live microorganisms is now considered as a paramount dietary approach to improve human health. Bacteria, yeast and fungi generally facilitate food fermentations. A considerable confusion exists on what type of fermented foods actually include live microorganisms and also the understanding their impact on the gut microbiome. Introducing the prospective advantageous microbes to the gastrointestinal tract by intake of microbe-having foods seem to be an easiest and suitable option.

Most microbes related to fermented foods may withstand digestion, reach intact to the gastrointestinal tract and eventually deliver health benefits. The vast benefits of such fermented foods has paved way in the development of synbiotic foods comprising of prebiotics and probiotic bacteria. Further novel functional foods with promising health effects are in huge demand among health conscious public and in the population influenced by changing eating patterns induced by urbanization.

Diet , Gut Microbiome and Microbiota



Sedentary lifestyle or various morbidities can impair the composition of human gut microbiota(HGM) , eventually contributing to metabolic disorders ,gut diseases and neurological diseases. A diversified healthy diet with restricted refined grains, highly processed foods and sugars should augment health and provide ideal nutrient intake. Although global consumption of fruits, vegetables, nuts and legumes has increased with a decreased consumption of red meat and sugar among health conscious consumers, processed foods still continue to be a part of diet. Dietary modification can prevent, treat, or ease some of the obesity, celiac disease or gastrointestinal(GI) disorders associated symptoms and improve general health.

Microbiome implies to the sum total microbial genome in a particular ecology, whereas microbiota is the community of microbes .A microbiome originating from a normal healthy gut is complex and consists a highly diverse collection of microorganisms (approximately 100 trillion)consisting of bacteria, viruses, fungi, parasites, viruses, protozoa, bacteriophages , archaea and other tiny organisms . The number of other bacterial cells are indicated by "human microbiome project" as approximately 10 times the number of human cells in the body, with more than 1000 different metabolically active bacteria (*Bacteroides* and *Firmicutes* being the main dominant flora) .This flora ably works with the host immune system , resisting stress associated changes and thus strongly impacting control of energy metabolism, metabolic functions , immune system , nutrition and intestinal functions of the host . Additionally, microbial residues of exopolysaccharides , building blocks of microbes , proteins, or metabolites, may influence human health with direct or indirect interaction with human physiology and gut microbiome. There is an ever-growing research with commercial concern in several aspects of prebiotic and probiotic functional foods to neutralize "dysbiosis" by substituting unfavourable microflora with favorable ones .

Various conditions like obesity, autoimmune diseases, and infections may cause predominance of harmful bacteria. Probiotics with its direct interaction with immune cells may maintain the immune balance in the GI tract, thus can be regarded as potential adjuvant therapy . There has been recent advancements in significant treatment of neurodegenerative illnesses owing to microbiome gut–brain axis . Quick gut lumen communication with brain after meals and the contribution of microbiota in homeostasis regulation with suitable signaling has been evidenced by research with the finding of "neuropod cells". An encouraging association has been reported in mothers consuming fermented foods and sleep period among year-old infants , attributed to variation in mothers gut microflora . The conversion of dietary flavonoids is determined by gut microbiota and its interaction with intestinal microbiota may not only regulate the HGM composition and functional enhancement of probiotics but it significantly increases the bioavailability and bioactivity of flavonoids thus regulating the intestinal diseases.

Primary and secondary metabolites , considered as bioactive compounds are formed during fermentation process, which include antibiotics, antimicrobial peptides, alcohol ,carbon dioxide, vitamins like folates and organic acids such as short chain fatty acids (SCFAs) (acetate, butyrate, propionate). Bioactive molecules with its therapeutic potential, are reported to be advantageous to the gut by improving the intestinal barrier, inhibiting production and subsequent development of pathogens, toxic elements and help prevent metabolic disorders. Dairy and non-dairy fermented foods may act as carriers of probiotics, prebiotics, and/or bioactive compounds (phenolic compounds, antioxidants, bioactive peptides, etc.) and significantly impact the gut microbiota . Biotics that include probiotics, prebiotics, postbiotics, synbiotics, paraprobiotics, oncobiotics, pharmabiotics and psychobiotics are emerging in recent years and have gained much attention .

Food Fermentation: ***Benefits, changes brought about in food and types of food fermentation***

Food fermentation is an ancient practice known to human kind around the globe. Derived from the Latin term “fermentum”, fermentation when applied to foods has a much broader implication as against its biochemical description as “an ATP-generating process with organic compounds acting together as electron donors and acceptors”. Foods made by preferred microbial growth along with enzymatic alterations of food components are referred as fermented foods. Approximately over 5,000 types of fermented foods and beverages are globally produced and consumed from diverse raw materials like roots and tubers, vegetables, cereals, legumes, dairy products, fish, meat and fruits Fermentation is much used in the making of probiotics, prebiotics and synbiotics containing great microbial viability and functionality.

Benefits of food fermentation:

Fermented foods and ingredients has appealed interest as they can support HGM , thus encouraging human well-being. Meeting the expectations of consumers to make available functional foods with added benefits, is a big challenge to food science and technology. Food fermentation considered as an ancient biotechnology, is considered as a good method for augmenting nutritional and sensory qualities. Consumption of fermented foods is promoted in Indian food pyramid affirming their significance in actual human nutrition framework.

Fermentation provide an assured guarantee to improve quality, organoleptic properties, nutritional , biological value of perishable foods with breakdown of large organic molecules into simpler ones via microbial action. The characteristic taste, flavor, color, consistency, appearance, texture, improved vitamins, essential amino acids, proteins, reduced anti-nutrients, extended shelf life and functional properties and health benefits due to microbial assimilation, actions of enzymes and of released metabolites. Fermented foods can be considered as a dietary form of living organisms.

Generally lactic acid bacteria (LAB) like *Lactobacillus, Streptococcus, Lactococcus Enterococcus* and *Bifidobacterium*) are used in processing of fermented food products in addition to molds (*Aspergillus oryzae, Aspergillus sojae, Penicillium roqueforti* and *Penicillium chrysogenum*) and yeasts (e.g. *Saccharomyces cerevisiae, Andida krusei* and *Candida humilis*). Fermentation is brought about by mono or mixed cultures of bacteria, fungi and yeasts, which act parallel or sequentially altering dominant microbiota. Yeast enzymes convert sugars and starches to alcohol with proteins converting to peptides/amino acids. The actions by microbes or enzymes tend to ferment food and causing desirable biochemical changes that significantly modifies the food. For eg; degradation of complex phytochemical compounds to smaller ones, release of more bioactive polyphenols prove advantageous for the microbiome, reduce inflammatory reactions and cytokine production. The plausible mechanisms for additional benefits of fermentation include reduction of free radicals or its neutralized effects and subsequent reduction in oxidative stress, controlling antioxidant enzyme activity and increase in activity of immune system. Reduced occurrence of asthma and atopic dermatitis, improvement in fasting blood glucose and other symptoms of metabolic syndrome were observed in overweight and obese Korean adults consuming kimchi and other fermented vegetables. Similarly, intake of phytoestrogens and bioactive peptides rich fermented soybean foods showed reduced dangers of Type 2 diabetes, hypertension with improved serum triglyceride levels in Japanese adults. Strong evidence in improvement of lactose tolerance with yogurt, owing to microbial release of β-galactosidase has been documented. The nature of substrate (legumes,cereal and pseudocereals) utilized in fermentation is of significant importance in the ideal delivery of bioactive substances in human nutrition.

Changes on fermentation in food: *microbiological activities, biochemical modifications and health impact*

Fermentation is a natural way of improving nutritional quality and organoleptic food properties . Making informed food choices and the acceptance of novel foods is influenced by sensory properties of food, thus the prospects of fermentation in creating edible and pleasant flavors and textures are of great relevance in changing eating habits. Changes happening during the fermentation that includes chemical transformation of primary compounds to new secondary metabolites can be categorized into 5 stages (Fig: 1). Stage 1 results in transformation of sugars to end products like carbon dioxide, simpler acids and alcohols that further enhances the nutrient bioavailability. Second stage, leads to hydrolysis of polymers with enzymatic synthesis of bioactive compounds like antioxidants. In third stage, there is enhanced bioavailability of phytochemicals and minerals with higher amounts of vitamins and amino acids. Stage 4 results in degradation of the toxic and anti-nutritional factors. Eventually, promoters get synthesized for improving absorption and uptake of compounds.

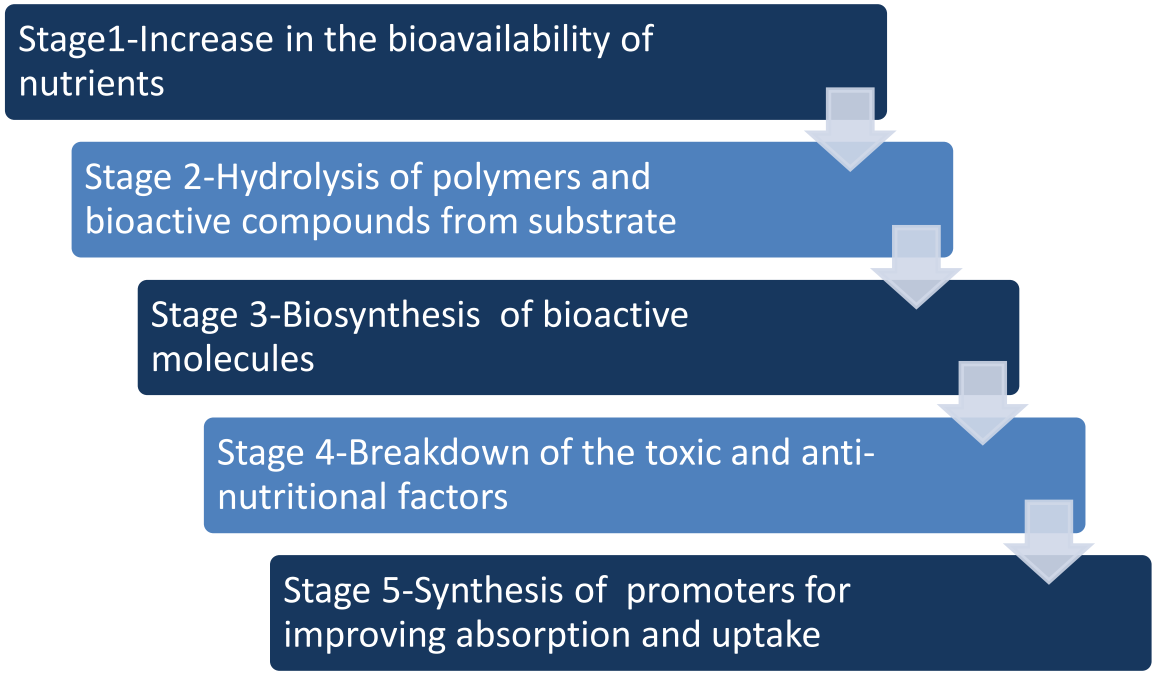


Fig.1. Five stages indicating changes during food fermentation.

Source: R. B. Cuvas-Limon *et al*, (2021).

Changes on fermentation in food that occur are as follows:

1. Food containing high fiber , toxins, anti-nutritional factors(ANFs) such as lectins, phytic , oxalic acids, tannins, raffinose, alkaloids, pyrimidine glycosides (e.g. vicine and convicine), trypsin and protease inhibitors ; that chelate metal ions , decrease digestibility and interfere with nutrient bioavailability, are likely to be changed to nutritionally enhanced and palatable foods via fermentation.
2. Substantial enhancement in protein digestibility can be brought about by fermentation.
3. Reduction in cyanide levels in leaves and the roots of staple cassava plant to acceptable levels and the removal of flatulence factors in legumes on its fermentation has been reported.
4. Fermentation may result in production of biotransforming phytochemicals from dietary polyphenols with a potential to trigger favorable changes to stimulate microbial development .

5. The Short chain fatty acids (SCFAs) produced on fermentation, especially butyrate, are responsible to maintain integrity of the intestinal mucosa, decreasing development of colon cancer risk by decreasing cell multiplication, encouraging cell differentiation and maturation of the enteric neurons. They are also recognized as important immune system modulators.
6. *De novo* synthesis of pleasant textures and flavors can be obtained by microbial fermentation eg: diverse cheese textures , chocolate flavor, soy sauce, beverages, condiments, etc. Fermentation synthesizes and buildup volatile and non-volatile aroma-active compounds, associated with tastes like sweet, bitter, sour, umami and salty. The attributing factors for changes in flavor may be due to the production of amines ,alcohol, aldehydes, esters, fatty acids (mainly volatile species), phenols, ketones, lactones, terpenes, organic acids, thiophene, diacetyl , sulfur compounds, pyrazines and other nitrogenous complexes .
7. Fermentation conditions along with microbial activities either with changes in pH, nutrient competition or by releasing preservatives having anti-fungal or anti-microbial properties in fermented foods may lessen the microbial count of pathogenic organisms.
8. Fermentation increases the release of bioactive substances (Table 1) from numerous raw material and functional biomolecules ,that constitute LAB/yeast metabolism.
9. Yogurt and fermented milk has high in vitro antioxidant characteristics, owing to formation of biopeptides on milk proteins proteolysis (usually of α-lactalbumin, β-lactoglobulin and α-casein)
10. Microbial fermentation has also enabled synthesis of GABA (g- Aminobutyric acid), principal inhibiting neurotransmitter of the central nervous system with a role to play in induction of antihypertension, prevention of diabetes, diuresis and tranquilizer effects .
11. As a food-based option, fermented foods can help meet folic acid demand of at-risk populations in dealing with global deficiency problem.
12. Fermentation reduces the energy required for cooking
13. As compared to its raw equivalents, fermented products are usually safer for consumption.
14. Extended shelf life of most fermented foods leads to the reduced food loss thereby improving efficiency and resource use of food production.

Table 1. Functional properties related to bioactive substances in fermented foods.

Functional Attributes	**Bioactive substances**	**Microbes**	**Fermented foods released in**	**Health aspects**
Antimicrobial Properties	Antimicrobial compounds Bacteriocin Pediocin Nisin Z	LAB *Lactococcus lactis* BH5, Leuconostoc *citreum* GJ7, *Pediococcus pentosaceus.* *Lactococcus lactis.* *Weisella cibaria.*	Vegetables Milk Kimchi Dahi (India) Fermented cabbage product	GI tract protection , Anticancer effects Antiviral effects
Antioxidant Properties	Antioxidants compounds, Peptides, Enzymes.	*Bacillus, Lactobacillus, Bifidobacterium*	Asian fermented soybean foods Soybean food of India and Nepal	Modulation of circulatory oxidative stress Protection of cells against carcinogen-induced damage . Anti-inflammatory Anticancerous
Immunomodulatory Properties	Amino acids ,Bioactive peptides	Proteolytic microorganisms (*Bacillus*), LAB.	Fermented beverages	Energy balance maintained Contribute to antioxidant effects , Anti-inflammatory effects
Inhibitory properties	Amino acids, Bioactive peptides	Microbial enzyme (ACE)	Kefir Koumiss Yogurt	Minimize the risk of developing chronic or degenerative diseases.
Enhancement of metabolic functions	Lipases, Proteases, Esterases, Amylases b-galactosidase, Lactase	LAB, *Bifidobacterium.*	Fermented beverages	Help to improve the metabolism ,Improve nutrient absorption ,Mitigate lactose intolerance
Production of	Propionate,	LAB	Fermented	Provides 60–70% of the

SCFAs	Acetate, Butyrate.		beverages	energy needed by the colon, muscle and brain cells. Therapeutic effects: Anti-inflammatory bowel , Anti-colon cancer ,aids in prevention of heart diseases ,management of obesity and diabetes.
Production of vitamins	Vitamin B12, Vitamin K, Vitamin B6, Cobalamin, Folate, Thiamin.	*Bifidobacterium, Lactobacillus.*	Fermented beverages	Enables DNA synthesis , Fights cardiovascular disease

Source: R. B. Cuvas-Limon *et al*, (2021).

Types of food fermentation:

Fermented foods are normally referred to as those processed by uncontrolled fermentation (natural) or controlled using starter cultures. It results in enzymatic changes of primary and secondary food components with cost-efficient bioprocess. Food fermentation is impacted by many factors, like substrate composition, fermenting microorganisms, food treatment and duration of fermentation whilst processing. Depending on the type of the matrices (substrate) exposed to fermentation, there exists nine main categories of fermented foods: (1) fermented cereals; (2) fermented legumes; (3) fermented vegetables and bamboo shoots ; (4) fermented roots/tubers; (5) fermented milk products; (6) fermented and preserved meat products; (7) fermented, dried and smoked fish products; (8) alcoholic beverages; and (9) miscellaneous fermented products. To initiate any microbial metabolism, the raw material used must be rich in chemical composition, together with high concentration of mono and di-saccharides and minerals with no inhibitors like toxins that may inhibit the microbial growth. Environmental conditions also impact the fermentation process.

Food fermentations are classified either based on added or naturally present starter cultures as **controlled** or **uncontrolled** (Natural/ spontaneous) fermentation respectively and based on the product produced. Based on the dominant metabolic process, three fermentation processes include: acidic, alkaline, and alcoholic fermentation, each contributing in a unique way. Acid fermented products or alcoholic beverages are widely documented for its nutritional and health benefits as compared to alkaline fermented foods (AFFs), thus the focus here will be on AFFs. Active research on "**alkaline food fermentation**" commenced in the late 1980s, while lactic acid fermentation was popular with detailed scientific research way back in 1950.

1. **Acidic fermentation**: is primarily done by LAB, acetic acid bacteria (AAB) and yeast with lactic acid as main metabolite along with different types of organic acids, thus called as LAB fermentation. Curd/ Yogurt and sourdoughs are produced by lactic acid and yeast fermentation respectively. AAB (mainly *Acetobacter*) oxidize carbohydrates, alcohols and sugar alcohols (polyhydric alcohols or polyols) to their equivalent primary product acetic acid, organic acids, ketones and aldehydes in oxidative fermentation.
2. **Alkaline fermentation** often occurs on seed fermentation and carried out by diverse species of *Bacillus* and fungi Eg. AFFs such as natto ,dawadawa, bikalga, ugba,,kinema, iru ogiri and thua-nao.
3. **Alcoholic fermentation**, with AAB and yeasts as predominating organisms primarily yields ethanol and CO_2. Eg. alcoholic beverages such as beer, wine, cider.

Alkaline-Fermented Foods: ***Nutritional significance of AFFs***

Alkaline-fermented foods made from various raw ingredients, are a lesser known food product in developing and a few developed countries. In alkaline-fermentation the pH of the substrate (usually protein-rich foods) increases to alkaline values (pH 8–10). This fermentation is either due to alkali-treatment during production or due to protein degradation/hydrolysis (proteolysis) to peptides, amino acids (essential) and further degradation of amino acids to ammonia by dominant *Bacillus spp*. The increase in pH contributes to a strong ammoniacal smell which can be controlled by restricting the bacterial growth and metabolism without hindering the action of flavor producing proteolytic enzymes. Holding of fermented beans at appropriately low temperature during maturation can also enable this restriction and is also possible by adding glycerol as humectant to partially fermented beans or by limiting oxygen supply. Proteolysis is considered as the main metabolic activity in alkaline fermentation.

Traditionally, AFFs are usually produced using spontaneous fermentation (dependent on existing autochthonous or native microorganism of raw substrate and of immediate environment) but can also be developed by controlled fermentation. Predominant microorganisms in AFFs include *Bacillus subtilis, B. subtilis var. natto, B. cereus, B. megaterium, B. thuringiensis, B. endophyticus, B. licheniformis, B. borstelensis, B. pumilus, B. coagulans, B. circulans, B. amyloliquefaciens, B. firmus, B. megaterium, Paenibacillus polymyxa, Lysinibacillus sphaericus* and *Lysinibacillus fusiformis*, in addition to the involvement of secondary microorganisms (*LAB, staphylococci* and *micrococci*). *Bacillus spp.* are used as probiotic in humans.

AFFs as functional foods: *Nutritional significance and health benefits of AFFs*

Plant-based substrates are usually used with soybean being the most common in AFFs. Textural and color changes of AFFs on softening are their important features. Softening of plant based foods leading to the desired texture occur due to hydrolysis of pectin and protein by pectic and proteolytic enzymes. Browning reactions (non-enzymic) which involves amino groups of amino acids and the non-reducing sugar components and microbial activity may contribute to the color development. Widespread production of whitish sticky mucilaginous polymers assumed to be polysaccharides are the characteristics of many alkaline fermentations. AFFs can immensely achieve dietary needs in communities producing and consuming them. Alkaline fermentations are known for two important features as follows:

1. **Hydrolysis of macronutrient molecules** i.e. of complex carbohydrates like oligosaccharides, proteins and fats by the enzymes produced from predominant bacteria, thereby augmenting nutrient bioavailability and digestibility as compared to unfermented substrate. For example, fermentation with B. *subtilis* can effectively hydrolyze soybean proteins and polysaccharides into low-molecular-weight, water-soluble products requiring minimal subsequent degradation by enzymes from GIT . Increased levels of non-protein, soluble nitrogen, free amino acids with increased concentration of methionine, cysteine isoleucine, phenylalaline, leucine, tyrosine and even lysine which is a limiting amino acids in plant sources are reported in alkaline fermentations.

Most plant based sources used for AFFs are abundant in carbohydrates with non-digestible oligosaccharides in large quantity being such as galactamannan, stachyose, raffinose, verbascose, sucrose and arabinogalactan. They act as functional prebiotics and enable the action of beneficial microbes. Some of these non-digestible carbohydrates in humans cause flatulence and abdominal distension. Fermenting microorganisms hydrolyze starch and non-digestible carbohydrates into readily digestible sugars (melibiose, fructose, galactose) and additionally positively influencing product's texture due to tissues softening. Enzymes like galactosidase , amylase, galactanase, fructofuranosidase and glucosidase produced on alkaline fermentation by *Bacillus spp.* degrade carbohydrates.

Alkaline fermentation also results in the change of crude fat and fatty acid profiles attributed to the lipolytic activity of dominating species of microbes. Most studies have reported increase in free fatty acid levels with very few reporting contradicting results. The composition of the free fatty acids like monounsaturated fatty acids, saturated fatty acids and polyunsaturated fatty acids varies. The presence of lignoceric, stearic, palmitic arachidic, behenic, linolenic, myristic acids , palmitoleic,and gadoleic acids along with linoleic and oleic acids in high amounts have been reported in AFFs.

Enzymatic degradation: This results in substantial reduction of allergens, ANFs and toxic components that occur naturally in the raw food substrate, thus improving the palatability of toxic raw material, inedible or difficult to digest foods. Fermentation brings about improvement in the nutritional value of foods by the action of protease and amylase enzymes on protein and insoluble sugar.

The most popular alkaline-fermented soybean products is Natto that has distinct odor, flavor and stringy material (fructan and glutamic acid based polypeptide) . Nattokinase which is a polypeptide of 27 amino acid residues is present in Natto. During natto production lipids, protein and minerals like calcium iron and zinc and tend to increase with decreased lipid content. Another remarkable compositional change in natto is the significant rise in vitamin K content , correlated to synthesis of vitamin K2 (menaquinone-7 or MK-7) by *B. subtilis natto* . A positive correlation has been reported on bone density with increased consumption of natto in Japanese females with increase in ϒ-carboxylated osteocalcin and serum vitamin K which are bone mineralization facilitators. Fermentation also revealed 3-fold increase in riboflavin and thiamine content with five times increase in vitamin B12 levels in comparison to the non-fermented substrate. Fermented soybeans are abundant in an amino acid polymer gamma-polyglutamic acid (PGA), which is responsible for imparting sticky texture to the product, acting as dietary fiber responsible for reduced levels of serum cholesterol and increase in absorption of calcium. Isoflavone genestein, a chemo preventive agent in cancer is also present in high amounts in fermented soyabeans

Formation of aroma compounds in AFFs:

Flavor of traditional AFFs has been mainly attributed to various volatile compounds viz: Pyrazines (acetoin, tetramethylpyrazine, 2,5–dimethylpyrazine,, 2-decanone, 3-methylbutanal, ethyl linoleate, , 3,5-dimethylphenylmethanol, , chlorobenzene), aldehydes, , esters, ketones, alcohols, alkanes, acids, alkenes, sulphurs, benzenes, amines, phenols, furans groups and pyridines formed during fermentation by the metabolic activities of *Bacillus* spp. The amino acid, particularly glutamate as well as peptides may lead to flavor enhancement. Free fatty acids are positively known to influence the development of characteristic flavors in food, but rancidity may be caused due to their their high levels . Pyrazines formed on heat treatment due to reactions between amino acids and sugars during the alkaline fermentation contributes to the flavor .However, sun-drying of AFF products may cause loss of some volatile aroma compounds. The data on volatile compounds produced in legumes by controlled microbial alkaline fermentation may help in deciding for the future choice of starter cultures.

Food safety of products by alkaline fermentation

Alkaline-fermentation are generally known to produce safe products. For most AFFs, raw materials take a long time to cook before fermentation (up to 40 h), thus contributing in removal of pathogenic bacteria, usually non-spore–forming. In addition, the AFFs are well-preserved and stable as result of dominant microorganism's antimicrobial effects on harmful bacteria and molds. Isolation of *B. subtilis* and *B. pumilus* from AFF showed inhibition and inactivation of Gram-positive as well as Gram-negative bacteria like *Micrococcus luteus Bacillus cereus, Staphylococcus aureus, , Listeria monocytogenes Enterococus facium, , Salmonella typhimurium, Escherichia coli, Shigella dysenteriae, Yersinia enterocolitica*, and *Aspergillus* mold. *Bacillus* strains isolated from some AFF is found to release antifungals (iturin A, surfactin). Further the free ammonia and high pH of AFFs does allow the growth of spoilage microorganisms .

Although AFFs may be generally thought as safe, they can at times be risky for food borne disease, a usual problem in all types of traditionally fermented foods. Further with household production using basic equipment under unhygienic conditions and hardly any training on Good Manufacturing Practices (GMP), can contribute to unsafe AFFs, persistently recontaminating the fermented seeds. Thus positive actions like adequate GMP guideline based training on hygienic conditions and hazard analysis critical control point (HACCP), better constructed equipment like improved fermentors easy to maintain, sanitation and cost effective operating cost and production of desirable starter cultures for controlled fermentation can improve the safety of traditional AFFs .

Spontaneous Versus Controlled Fermentation:

Fermentation can be spontaneous with uninoculated cultures (**Natural /Uncontrolled fermentation**) or can be facilitated by addition of starter cultures (**Controlled fermentation**).

Spontaneous Fermentation

Natural fermentation which is a spontaneous phenomenon occurs with natural presence of a microbial consortium (autochthonous) in either animal or vegetable substrates to initiate fermentation. The microorganisms may also be present in the equipment or manufacturing environment.

During the progress of fermentation, microorganisms may act in a sequential manner or parallel in tune with the fluctuating dominant flora. *Leuconostoc, Streptococcus, Micrococcus, Lactobacillus, Pediococcus* and *Bacillus* are the usual fermenting bacterial species. *Aspergillus, Cladosporium, Paecilomyces,Penicillium, Fusarium* and *Trichothecium* are the common genera of fermentation leading fungi. Fermentation of various cereals and legume based substrates is by yeasts , Lactic acid bacteria (LAB) and/or fungi to produce variety of food by natural fermentation .They classically result in products with comparable microbes (with same species as well), irrespective of their origin . For instance, fermentations of cabbage and other green leafy vegetable commence with *Leuconostoc mesenteroides* with subsequent action by species of *Lactiplantibacillus* and *Levilactobacillus brevis*, irrespective of the product name as sauerkraut in North America and Europe, sinki of Nepal, Korean kimchi or Chinese suan-cai . Such a fermentation microbiota which is highly reproducible in spontaneous plant based fermentations reveals a steady association between organisms with raw materials. With such consistency in the classic techniques, environments , raw materials and factors such as pH, concentrations of salt, atmosphere and other controllable measures are in place during food fermentation, any erratic events likely to cause fermentation failure, are relatively rare . Spontaneous fermentations can also create a potential for strain isolation having industrially friendly attributes, like unique flavor production. Integration of such strains can enhance the products flavour in mixed fermentations apart from achieving better control on the process. With the attractiveness of spontaneous fermentations, it becomes vital to characterize and know the fermentation dynamics for regulation on the process for achieving improved quality attributes.

Since a complex microbial consortium influence spontaneous fermentations , their individual genotypic and phenotypic nature is dependent on the community assembly, intensely influenced by abiotic and biotic factors ,determining the fermentation network. Despite spontaneous fermentations great utility in safeguarding plant based foods, chances of fermentation failure, with respect to nutrient composition , incorrect inhibition of spoilage organisms or pathogens and the dangerous risks associated with their implantation/domination for humans and unfavorable sensory traits are significant. With heterogeneous microbiota involved in spontaneous fermentation, the chances of finding toxic by-products (ethyl carbamate, mycotoxins,biogenic amines) releasing microbial pathogens and/or strains that can impact the fermented products safety. Thus spontaneous uncontrolled fermentations is a big challenge to ensure food safety and quality.

Controlled fermentation

Safety of fermented food associated with microbes in spontaneous fermentation can be conciliated using personalized specific starter cultures to mimic associated protechnological microbial diversity. Selected starter cultures are used to bring about fermentation, even the naturally existing cultures regarded as probiotics that are isolated from fermented products. They may speed up the fermentation process, warranting increased shelf life of the fermented foods with enhanced functional and organoleptic features, provide uniform quality products and render health benefits.

The selection of starter culture(s) for use in production of functional foods are based on key criteria like antimicrobial properties, probiotic properties, antioxidant, fibrinolytic activity, production of peptides , poly-glutamic acid , destruction of ANFs, etc. Appropriate starter cultures may enhance product quality by their accelerated metabolic activities with appropriate organoleptic properties, more likely improved and safe fermentations with reduced hygienic risks. keystone towards safety and quality assurance of fermentation is the use of starter culture technology as it may reduce food-borne disease risks , offer personalized nutrition and help achieve new health targets. This technology may sustainably extend shelf life, especially of traditional, artisanal, typical, biodynamic and organic productions, yield increase, and food security .

Functional foods :Prebiotics , probiotics , synbiotics and postbiotics in food fermentation

There is a rapid and continuous interest in functional foods globally. Synbiotic foods, owing to their health benefits are playing a significant vital role in nutrition . There is great demand for the food creations having dietary fibers, fatty acids, vitamins, proteins, minerals and flavonoids, either naturally present or added .

Definitions :

International Scientific Association for Probiotics and Prebiotics (ISAPP),2010 defines **prebiotics** as "selectively fermented ingredients resulting in specific changes in the composition and/or activity of the gastrointestinal microbiota , thus conferring benefit(s) upon host health" . The definition of prebiotic was quite recently revised as "*a substrate that is selectively utilized by host microorganisms conferring a health benefit*"

Probiotics can be defined as "*live microorganisms that, when administered in adequate amounts, confer a health benefit on the host.*"

Synbiotics are a combination of prebiotics and probiotics , having a synergistic effect by inhibiting the growth of pathogenic bacteria and enhancing the growth of beneficial organisms

Post-biotics are bioactive soluble by-products released either by the metabolic processes of probiotics or through their lysis. A technical definition of post-biotics, by ISAPP is "*preparation of inanimate microorganisms and/or their components that confer a health benefit on the host*"

Prebiotics

Prebiotics are categorically functional foods defined as the non-digestible food ingredients. Their consumption as dietary ingredients, may positively influence the human gut microbiota (HGM) composition and metabolic functions in small intestine and colon . Different bacteria may be modified by diverse prebiotics at strain or species level.

Since prebiotics cannot be degraded by gut digestive enzymes, it reaches intact to the large intestine . Therein, thus serving as a substrate and allowing gut probiotic bacteria to grow by acting as a source of energy or carbon. The resulting in increased probiotic count. Prebiotics act synergistically with probiotics with symbiotic action resulting in optimal repopulation of the flora and suppressing the pathogens, which subsequently augment the immune system and gastrointestinal functions. Intestinal optimal pH is essential for the probiotics existence and is maintained by prebiotics. Peristalsis stimulation and reduction in gas formation are also prebiotic functions in addition to their role in increasing calcium and magnesium absorption and improving levels of blood glucose and plasma lipids .Diets with prebiotics, besides increasing the HGM diversity, influence microbial metabolic activities and the synthesis of end products like SCFA's, branched-chain fatty acids,peptides, organic acids, ammonia, phenolic compounds ,amines and gases . Prebiotics are also extensively studied as protectants for encapsulation of probiotic strains to enhance the probiotic viability

Plant based prebiotics

- Inulin
- Fructooligosaccharides (FOS)
- Galactooligosaccharides (GOS)
- Trans-galactooligosaccharides (TOS)
- Mannan- oligosaccharides (MOS)
- Xylo-oligosaccharides (XOS)
- Lactulose
- Dietary fiber(soluble and insoluble)
- Gums
- Lafinose

Dietary Sources

- Garlic, onions, guar gum,shallots, leeks,asparagus, Jerusalem artichokes, spinach, chicory root , agave, jicama, peas, lentils, beans, raw oats, soyabeans, unrefined wheat, unrefined barley , yacon and bananas

Figure 2 Sources of prebiotics

Most of the prebiotics are plant products, well recognized being carbohydrates (examples in fig.2) whereas certain lipids, some proteins and peptides are possible prebiotics . The effect of prebiotic at specific site in the GI tract and its benefits to host is determined by chain length of the chemical short chain, middle chain or the long-chain). Breast milk oligosaccharides are reported as original prebiotic and dietary sources like plant cell wall polysaccharides and gentio-oligosaccharides have also gained attention as novel prebiotics in addition to marine poly-/oligo-saccharides, sugar alcohols (xylitol, erythritol,sorbitol, isomalt, mannitol, lactitol, polyglycitols, maltitol) .Algae and microorganisms are extensively studied for their prebiotic potential ,the most familiar algal polysaccharides of brown algae being laminarins, alginates and fucoidans , carrageenans and agar (from red algae) and ulvans (from green algae) . Food-grade bacteria are prospective sources of non-digestible carbohydrates and bifidogenic prebiotics. Species of *Lactobacillus*, *Streptococcus*, and *Leuconostoc* are known to produce oligosaccharides like dextran, levan and mucan and hetero or homo-polysaccharides.

A **dietary substance** to fulfill prebiotic definition must meet specific criterias by *in vitro* and *in vivo* tests of non-digestibility (resisting enzymatic digestion, intestinal absorption and low gastric acid pH); undergo fermentation using intestinal microbiota and stimulate the growth and activity of intestinal bacteria selectively without getting affected by conditions of food processing. Prebiotics should be accessible in the intestine for bacterial action without chemically altered, and non-degraded .**Fructooligosaccharides, lactulose , galactooligosaccharides** and **non-digestible** carbohydrates are able to meet these criterias as prebiotics .

Probiotics:

The science of probiotics covers features in microbiology field to food processing. Intake of probiotics in sufficient amounts may provide health benefits to the host . They control the functioning of intestinal epithelial cells, lessen gastrointestinal infections, improve lactose metabolism and show antimutagenic and anti-carcinogenic properties. Supplementation of Probiotic has a potential to diminish the intensity or reduce the period of the infections with its stimulating effects on immune system. Right type and levels of exogenous dietary supplementation of probiotics species/strain can briefly colonize the GIT with stability in the composition of native microflora, thereby reestablishing dynamic physiological function of a symbiotic flora. The multitude of health benefits of probiotics, are through their biological mechanisms in the body.

Food based probiotic functional foods can be divided into **dairy** (cheeses, creams, yogurts, ice cream, milk, acidified milks) and **non-dairy products** (meat and meat products, cereal grain based bread or other fiber snacks, chocolates, fruit juices, other fruit preparations). It is vital to preserve the efficacy of probiotic bacteria and enhance its viability during the development of functional foods. As per International standards, probiotic functional products claiming health benefits should have a minimum count of 10^6 -10^7 CFU/g viable probiotic bacteria, when sold. To protect probiotic survival and viability and help deliver bioactive ingredients both to the food matrix itself and to the GI tract several approaches have been undertaken like immobilization technologies , composite carrier matrix systems, synbiotic development, adding osmo, thermo, or cryo-protectants to the drying matrix or growth media, lyophilization , microencapsulation(ME) through food matrix modifications and manipulation of the living cells adaptive mechanisms for survival under stressful conditions .

Lactobacillus, Lactococcus, Bacillus, Streptococcus, Bifidobacterium, Pediococcus, and *Propionibacterium* are familiar probiotic species with *Lactobacillus* and *Bifidobacterium* being the most common. Yeast probiotics include *Saccharomyces carisbergensis* ,*S. cerevisiae* and *S. boulardii* and *Aspergillus niger* and *A. oryzae* are regarded as fungal probiotics. Probiotics are commercially available as functional foods, dietary supplements and medicinal probiotics and are usually orally administered. Probiotics have been linked with nutrients in competing with pathogens, inhibit/block pathogenic bacterial adhesion in the colonic lumen, and improve mucus production, thus enhancing the intestinal epithelial barrier for immune system stimulation .As a prophylactic regime , probiotic supplementation has proven advantageous in antibiotic-associated diarrhea (AAD) by enabling replenishment of the gut microflora , probiotics has been found to reduce allergies in children , decline *Helicobacter pylori* colonization in the stomach , manage relapse of some inflammatory bowel conditions , reduce the risk of certain cancers by secreting specific antioxidant and anticarcinogenic metabolites , proven useful in dealing with periodontitis and oral *Candida* infections. Probiotics with its bile salt hydrolase and other enzymatic activities are reported to decrease toxins and increase the bioavailability of nutrients in the body.

Probiotics-linked signaling of nerve functions facilitated by specific mechanisms in the central nervous system, can stimulate possible therapeutic actions on neuropsychiatric disorders and stress-induced diseases. Probiotics also support brain functioning, overcomes irritable bowel syndrome, reduce l serum low-density lipoprotein levels, manage yeast/ bacterial vaginal and urinary tract infections, prevent pancreatitis, advance pancreatic health, facilitate respiratory tract health, inhibit tumorigenesis and contribute to metabolic homeostasis to help in dealing with metabolic diseases like diabetes cardiovascular diseases and non-alcoholic fatty liver disease. Apart from its physiologic effects , the bioavailability of micronutrients can be enhanced by probiotics, probiotics may reduce ferric to ferrous ions thus increasing duodenal absorption of iron as well as calcium uptake and absorption. Probiotics are also involved in synthesis of vitamin D synthesis and its absorption.

Probiotic through their metabolic activity produce soluble factors, called **postbiotics** such as bioactive metabolites through non-specific mechanism such as SCFA’s, vitamins (Vit-B_2 and Vit-K_2) , enzymes, lactic acid, bacteriocins , ketones, methyl acetates, carbon disulfides and secrete pathogenic bacteria destroying antimicrobial peptides. Postbiotics, directly or indirectly exerts a favorable effect on the host, and the risks associated with their intake being minimal as they are devoid of live microorganisms.

Table 2. Probiotic products available commercially

Brand	Description	Producer
Actimel	Drinking yogurt with *L. casei Imunitass* as probiotic	Danone, France
Activia	Creamy yogurt having *Bifidus actiregularis*	Danone, France
Gefilus	An extensive range of LGG products	Valio, Finland
Hellus	Dairy products containing *L. fermentum* ME-3	Tallinna Piimatoööstuse AS, Estonia
Jovita Probiotisch	Cereals, fruit and probiotic yogurt blend	H&J Bruggen, Germany
Pohadka	PohadkaYogurt milk with probiotic cultures	Valašské Mezirˇícˇí Dairy, CzechRepublic
ProViva	Refreshing natural fruit drink and yogurt in many different flavors containing *L. plantarum*	Skåne mejerier, Sweden
Rela	Yogurts, cultured milks and juices with *L. reuteri*	Ingman Foods, Finland
Revital Active	Yogurt and drink yogurt with probiotics	Olma, Czech Republic
Snack Fibra	Snacks and bars having natural fibers , extra vitamins and minerals	Celigüeta, Spain
SOYosa	Soy and oat based variety products like refreshing drink and a probiotic soy oat yogurt	Bioferme, Finland
Soytreat	Kefir like product with six probiotics	Lifeway, USA
Yakult	Milk drink containing	Yakult, Japan
Yosa	Yogurt-like oat product flavored with natural fruits and berries containing probiotic bacteria (L. acidophilus, B. lactis)	Bioferme, Finland
Vitality	Yogurt with pre- and probiotics and omega-3	Müller, Germany
Vifit	Vifit Drink yogurts with LGG, vitamins, and minerals	Campina, the Netherlands

Source: Adapted from Siró, I. 2008. Appetite, 51, 456–467.

Table 3. Differences between prebiotics and probiotic

Categories	Prebiotics	Probiotic
Content	Non-digestible but selectively fermentable ingredients	Live microorganisms
Functions	Provide food for probiotics; increase number and improve activity of probiotics	Enhances the digestive tract of their host organisms' by its health enhancing properties

Health Benefits	Supports probiotics	Decrease the amount of pathogenic bacteria in the GIT thus improves GIT function; improves immune functions; prevents cellular damage from oxidative stress
Sources	Oatmeal, asparagus, Jerusalem artichokes, , onion, garlic , inulin, guar gum , bananas ,pulses, etc.	Isolated microbial cultures, yogurt, sauerkraut, yakult, miso soup, snack bars and fermented breakfast cereal, sourdough, soft cheeses, kombucha and kimchi
Side Effects	Increased fermentation resulting in increased gas production, bloatedness or bowel movement	Chances of sepsis on administering to immune compromised patients

Synbiotic approach towards functional foods:

Synbiotics with prebiotics and probiotics combination, inhibits pathogenic bacterial growth whereas augments the growth of beneficial organisms and thus displays a synergistic effect. A good synbiotic diet can strongly influence the gut microbiome. A well designed synbiotic formula should have probiotic encouraging prebiotics, predominantly facilitate their growth. Synbiotics are formulated to beneficially affect the host with improved survival of health-promoting probiotics and its implantation in colon by selectively stimulating the growth therein or their metabolism in addition to maintenance of the intestinal biostructure. Synbiotics should improve the gut microbial composition with production of metabolites and bioactive compounds with the concomitant inhibition of prospective pathogens residing in the GI tract. Synbiotics are reported to decrease the number of infections in postoperative patients, improve the outcome of irritable bowel disease(IBD), and present anticancer effects. Patients affected by small intestinal bacterial overgrowth (SIBO) showed better results on treatment with antibiotics and synbiotic supplementation (with *B. coagulans* and prebiotics) than antibiotics alone. Synbiotic are also reported to lower the quantity of unwanted metabolites, lessen the inactivation of nitrosamines and carcinogenic substances. Determining the best pre-probiotic combination warrants for a structured approach in individual and specific disease setting.

Difference Between Fermented Foods and Probiotics:

The practice of traditional food fermentation dates back to human history. The International Scientific Association for Probiotics and Prebiotics (ISAPP) redefined fermented foods and beverages in 2019 as foods made through desired microbial growth and enzymatic conversions of food components.

Fermented foods and beverages are at times labelled as “probiotic foods” or “contain probiotics” by the manufacturers in an attempt to communicate to consumers about the presence of health stimulating live microorganisms in the product. Term ‘probiotic’ should only be used when there is established health benefit offered by characterized live microorganisms, extending beyond any nutritional advantage of the food substrate. ‘Fermented food’ and ‘probiotics’ are the terms not to be used interchangeably. Some jurisdictions recognize several common species for which the term ‘probiotic’ can be used in foods, such as Health Canada recognizes more than 20 species of the *Lactobacillus* genus complex and *Bifidobacterium* provided they are delivered at a minimum of 10^9 CFU per serving. European Food and Safety Authority approves health claims of live yoghurt cultures, particularly their improved lactose digestion, essentially owing to the presence of the lactase enzyme in yoghurt cultures (*Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*). However, the label “contains probiotics” in fermented foods should only be used when the strains (regardless of one or more used) are defined to the strain level with known genome sequences and are present at in desirable numbers throughout the shelf-life of product.

In recent times, maximum of commercially sold fermented foods do not belong to the class of “probiotic fermented food”. Undefined microbial consortia are often found in fermented foods and beverages although at variable levels, with no demonstration of their potential health benefits. It is suggested that manufacturers indicate their product contains “live and active cultures” with assurance that food is not treated to remove or destroy the fermentation microorganisms. Further these microorganisms should be present at expected levels depending on the foods. Pasteurized fermented foods having no live microorganisms can be labelled as “foods made by fermentation”. Although microbial cultures organisms used for fermentations are characterized and identified at strain level, they are generally chosen based on their performance characteristics, like rapid acidification, substrate conversion, flavor and texture properties and not only on health associated functions.

Regulatory considerations for fermented foods

Food fermentation guidelines mainly concerned with food safety are covered in International regulations. Irrespective of strains developed by recombinant DNA technology or genetic modification, their safety needs to be established and use be regulated .There exists designations like 'Generally Recognized As Safe'(GRAS) in the USA or the 'Qualified Presumption of Safety'(QPS) designation assigned by the European Food and Safety Authority to groups of microorganisms. Further identification of vital microbial components in fermented foods has prospects to lead to new food labelling regulations. Regulations with regard to fermentation warrant that minimum prerequisites are met concerning to the contribution of specific microbial taxa. A few standards exist, commonly for cultured dairy foods. As per Codex Alimentarius, yoghurt manufacturing should use a combination of *S. thermophilus* and *L. delbrueckii* subsp. *bulgaricus* and kefir (fermented milk) should include *Lentilactobacillus kefiri* , lactose fermenting yeasts (*Kluyveromyces marxianus*) ,non-lactose fermenting yeasts (*Saccharomyces unisporus*, *S. cerevisiae* and *S.exiguus*) and species of *Leuconostoc*, *Lactococcus* and *Acetobacter*. With identification of newer fermenting microbes with beneficial effects similar standards could emerge like kombucha and water kefir.

Integrating fermented foods into dietary guidelines

Fermented foods would contribute to meet important macronutrients as well as supply large count of prospective beneficial microbes to the gastrointestinal tract. Recently, the concept of "shared core benefits" was introduced to explain how and why phylogenetically related organisms could deliver similar health benefits. Regular intake of fermented foods having live microorganisms should be promoted around the globe by health care professionals and should be included in dietary guidelines for all the age groups. The Indian food pyramid clearly encourages the intake of fermented foods testifying their significance in actual human nutrition. Many of the microbes in fermented foods are largely related to probiotic organisms as they often share similar molecular mechanisms, accountable for health supporting properties; even though by definition they cannot be considered probiotic,

Summary:

- There is a rapid interest in functional fermented foods and probiotic foods for their immense contribution to nutrition owing to their beneficial health effects. Nutritive value, quality and organoleptic properties of foods are enhanced by fermentation.
- Fermentation, a biotransformation process involves the added starter culture (in controlled fermentation) or indigenous microorganisms (in natural or uncontrolled fermentation) and the substrate as two prime factors in addition to inherent factors like temperature and the pH conditions. The presence of starter cultures or native microorganisms contribute to the probiotic functions of several fermented foods.
- Fermented food products contribute to an enriched indigenous dietary diversity offering sustainable food security. Depending on the type of microbes, fermentations can be categorized as bacterial or fungal. Based on the food used, it is referred to as fermented cereals, vegetables, fruits, legumes, roots/tubers, milk, meat, and fish products.
- Fermentation results in the production of metabolites (primary and secondary) by microbial action such as antibiotics, antimicrobial peptides (ACE inhibiting peptides}, carbon dioxide, alcohol, vitamins, folates, SCFAs, intracellular and extracellular polysaccharides of microbes, antioxidants compounds, complex compound degrading enzymes resulting in simple ones, bacteriocins, and cell wall components, all collectively known as postbiotics and are considered bioactive compounds. These components directly or indirectly can benefit the host.
- The properties of probiotic cultures are augmented by prebiotic constituents during the manufacture of probiotic foods. Prebiotics can escalate the nutraceutical properties of foods, facilitate the intestinal growth of beneficial bacteria and protect the probiotics in gastrointestinal tract. Individual outcome of the probiotics and prebiotics can be enriched by using them collectively as synbiotic.

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