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Biotechnological Production of Nutraceuticals and their Delivery in Different Foods Forms

Janifer Raj X, Ilaiyaraja N, Pal Murugan M, Chauhan OP and Ramana KV

Defence Food Research Laboratory, Defence Research and Development Organization, Mysore, Karnataka, India-570011
Corresponding author: janiferxavier@gmail.com

Abstract: Nutraceuticals provide the benefits of nutrients and pharmaceuticals together for disease prevention and therapy. Bioactive compounds are produced by plants, animals, fungi, algae and bacteria. Nutraceuticals are found to be beneficial for health related problems or diseases such as hypertension, oxidative stress, obesity, hypercholesterolemia, diabetes, fatigue and anxiety. Plant foods possess several components but their bioavailability is enhanced through biotechnological interventions using fermentations or enzymatic processes. The bioavailability is increased in fermentation through β-glucosidase mediated reactions produced by fermentative microorganisms. The benefits rendered by probiotics, prebiotics and synbiotics are enormous and they synergise health benefits of bioactive compounds produced by plants. Currently conjugated linoleic acid is produced by enzymatic transformations and lactic acid bacteria using less valued raw materials. Metabolic engineering has further broadened the vistas of nutraceuticals for large scale production in heterologous hosts in a cost effective manner using microorganisms and fermentations. Some important vitamins such as vitamin B_{12} and low calorie sweeteners i.e. tagatose, sorbitol, mannitol and L-alanine have been produced using microbial systems. In the current manuscript we described microbial bioprocess based processes for nutraceuticals production and their applications with some emphasis on waste or less valued food by-products for bioactive conversion and extraction.

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1. Introduction

Food is a major component of human life and is directly related to the status of health, prevention and treatment of various diseases right from the inception of existence, albeit the importance of food for enhancing the quality of prolonged life span has gained great importance worldwide, in the recent decades due to modernization and globalization. In this regard, nutraceuticals are preferred over the readily available synthetic pharmaceuticals. Nutraceuticals are combinations of nutrients and bioactive compounds that function together as foods and pharmaceuticals ensuring health and medical benefits in humans with a natural acceptance and safety. Plants, animals, microorganisms (whole or their products) that inhabit both terrestrial and aquatic environments are found to be useful as nutraceuticals. Very often the health promoting, disease preventing and therapeutic components of nutraceuticals are referred to as bioactive compounds. In some contexts the nutraceuticals are extracted or purified from the bulk food material and used in the form of capsules, tablets, powders, or liquid formulations seemingly giving the impression of pharmaceuticals. Microalgae are ingested as a whole-cell as it contains some of the bioactive compounds. With a distinguishing mode, a nutraceutical is a product purified from the food and generally sold in medicine form, not usually associated with normal foods. A large number of algal products derived from microalgae are marketed in the form of tablets, capsules, or liquid extracts. Consumption of nutraceuticals in the form of food also ensures convenience, ease of availability as well as their cost-effectiveness. According to recent reports, nutraceuticals are structurally and functionally defined specific bioactive molecules with defined medical and physiological effects along with pharmaceutical properties (Wang *et al.*, 2016). With the advent of recombinant DNA technology, progress made in the area of fermentation technology, down-stream processing and extraction technologies of a variety of nutraceuticals and their quality has been dramatically improved in treatment of health related problems. Using metabolically engineered microorganisms and tailored fermentation processes, nutraceuticals production in a relatively pure form, from readily available materials in large scale has become a reality (Fig 1). Nutraceuticals are found to improve conditions such as oxidative stress, depression, inflammation, osteoporosis, improvement of gastrointestinal health, hypercholesterolemia, cardiovascular diseases, hypertension, diabetes, cancer, hepatoprotective, immunomodulation, obesity and allergen related complications. In the current review the importance of nutraceuticals in human health and various food substrates that can be enriched with bioactive molecules produced by fermentation processes by valorization of cost effective agro-industrial wastes as substrates has been discussed. In addition the importance of biotechnological methods and organisms that play a major role in the bulk production of nutraceuticals, particularly from aquatic and marine sources has also been discussed.

2. Probiotics, Prebiotics, Synbiotics and Parabiotics

Probiotics are living microorganisms if ingested in adequate quantities that provide health benefits in the gastrointestinal system. The probiotic bacteria mainly include *Bifidobacteria, Lactobacilli, Streptococcus thermophilus*. A probiotic must be from human origin, non-pathogenic, suitable for food fermentation and food processing, stable or withstand acidic pH, and bile salt tolerant and able to improve gut health through immunodulation. Several species and strains of bacteria that belong to the genera *Lactobacillus, Bifidobacterium* and *Saccharomyces boulardii* are some of the probiotics widely used in the market as well as in food fermentations. Intake of *Lactobacillus rhamnosus* at $5X10^9$ cfu ml^{-1} for 28 consecutive days were found to reduce stress related symptoms in the form of reduced corticosterone level, altered GABA expression in the brain (Bravo *et al*., 2011). In clinical trials *Lactobacillus helveticus* and *Bifidobacterium longum* have shown to reduce anxiety in human volunteers (Messaoudi *et al*., 2011). Various ageing related problems in elderly people can be moderated with the use of probiotics such as *Lactobacillus rhamnosus, L. acidophilus* by suppressing *Clostridium difficile* in faeces. The yoghurt culture *L. delbrueki* sub sp *bulgaricus* and S*treptococcus thermophilus* could be used as symbiotics for fermented milk production while *Bifidobacterium animalis* sub sp *lactis* BB12, *B. bifidum* CCDM92 are some of the known probiotics with useful attributes. Several fermented products such as yoghurt, kimchi, kefir, sauerkraut, soy milk, and fermented soy products i.e., natto, miso, tempeh etc are well studied for their probiotic and prebiotic effects. Prebiotics are non-digestible oligosaccharidessuchasinulin,fructooligosaccharides,galactooligosaccharides, isomaltooligosaccharides which improve the metabolism and growth of probiotics. Prebiotic oligosaccharides such as isomalto-oligosaccharides and isofructo-oligosaccharides can be produced using transferases extracted from *Aspergillus foetidus*. These enzymes are specific for maltose and sucrose for synthesis of isooligosaccharides (Wang and Rakshit, 2000). The prebiotic components are also found in chicory roots (inulin), banana, tomato, and allium and fruits rich in oligosaccharides. Synbiotics are the combination of probiotics and prebiotics administered together for their synergistic activity in promoting gastrointestinal health by improving survival and adherence of administered live microbial supplements. Parabiotics are non-viable microbial cells or the disintegrated cell fragments or cell extracts of lactic acid bacteria or *Bifidobacteria* if administered orally or topically, in sufficient quantity would confer health benefits in the human body. The intracellular and extracellular bacterial components with immune-regulatory properties include peptidoglycan, lipoteichoic acid, extracellular phosphopolysaccharides, DNA/RNA and surface layer proteins (*s*-layer) secreted proteins, and various other cell-wall associated polysaccharides. The fragments of DNA motifs were found to induce immunoglobulin secretion and B-cell proliferation or inhibition of IL-8 secretion as noticed iHT-29 cells on stimulation with tumor necrosis factor-alpha. The genomic DNA motifs from lactic acid bacteria were found to participate in the process of immunoactivation of gut associated lymphoid tissue (Shigwedha *et al*., 2014).

3. Fermented Foods as Nutraceuticals

Ancient practices of food fermentations paved the way for modern discipline of food science and technology. Fermentations of food were mainly invented to preserve foods (enhance shelf life) and to enhance the nutritional quality, digestion and organoleptic properties. Several foods comprising vegetables, fruits, cereals, herbs, meat and fish were fermented with enhanced nutritional properties (Fanworth, 2004). The awareness about the health benefits of nutraceuticals is growing at a very fast pace.

3.1. Fermented Soybean Products

Soy milk was invented almost 2000 years back by An liu in China. Soy products contain isoflavones and phytosterols that are well established health promoters and are being used as nutraceuticals. Soy beans also contain high protein content, oligosaccharides such as stachyose and raffinose. Particularly, soymilk has been proved to support the growth of fermentative microorganisms as well various probiotic organisms. Fermentation of soy milk is carried out using probiotic bacteria such as *Lactobacillus* and *Bifidobacteria* which are associated with strong proteolytic acitivity and suitable to hydrolyze soy protein comprising glycinin and β–conglycinin. Lactic acid bacteria contain high levels of β-glucosidases to form isoflavone aglycones. The fermented soymilk is proved to be as good as fermented dairy products, during fermentation process it is enriched with bioavailable, bioactive compounds such as isoflavones, peptides and vitamin B content along with oligosaccharides. Isoflavones are plant origin dietary phytoestrogens that are investigated for their role in preventing hormone related diseases such as breast cancer, prostate cancer, osteoporosis and cardiovascular diseases. The isoflavones in the soybean are present in various conjugated forms known as aglycones. The glycoside form of isoflavones such as daidzin, genistin, is hydrolyzed to daidzein and genistein during soy bean soaking and fermentation. Apart from protein degradation into amino acids pool and supporting the microbial growth the proteinases of fermenting bacteria release angiotensin I-converting enzyme (ACE) inhibitory peptides that are useful to lower hypertension and improve conditions such as atherosclerosis, and function as anticancer, anti-diabetic, antiobesity, and improve gastrointestinal health. The bacterial communities present in soy milk have been shown to suppress growth of *Clostridium difficile* and other coliforms (Fig.2) (Ewe, 2015). Soybean peptides along with the contents of probiotic bacteria and isoflavones provide it the properties of nutraceuticals. Supplementation of soy milk with B vitamins, prebiotics such as inulin, fructo-oligosaccharides, mannitol, maltodextrin, nitrogen sources such as yeast extract, peptone, tryptone, caseitone and carbon sources such as sucrose, fructose, and lactose could enhance the growth of probiotics in soy milk in the form of nutraceuticals. Moreover, preparation of soy milk is cost effective and readily available. The fermented soy protein is easily digestible, reduces anti-nutritional factors, and improves calcium and vitamin B quantities. The trisaccharide raffinose and tetrasaccharide stachyose in soy milk are hydrolysed to D-galactose and sucrose by α-galactosidase excreted by microorganisms. An invertase hydrolyzes sucrose to fructose and glucose to be metabolized by the fermenting microflora. Hydrolysis of stachyose and raffinose is important to reduce the flatulence by soy products.

There are several soybean products in Asian countries fermented using yeast and fungi such as miso (Japan), sufu (China), and tempeh (Indonesia). Kefir and kimchi are well known fermented foods produced using lactic acid bacteria such as streptococci, lactobacilli, acetic acid bacteria and yeast. Kimchi is a traditional Korean fermented cabbage product and contains gamma amino butyric acid (GABA) which promotes gut health. Other GABA containing fermented foods are miso, sauerkraut, tempeh and yoghurt. GABA was proven to promote brain functioning, reduce anxiety, improve sleep and also be a neurotransmitter. *Rhizopus oligosporus* which is used in the production of tempeh is also known to produce β-glucosidases. It releases aglycons from isoflavones, as a result bioavailability, bioactivity and antioxidant properties of tempeh increase drastically. *Micrococcus luteus* isolated from tempeh further transforms the glycitein to a new isoflavone identified as factor 2 (6, 7, 4'-trihydroxyisoflavone) by o-methylation reactions. The factor was found to exhibit stronger antioxidant activity than other isoflavones and it was not in soybeans as such. It has been shown in the process of fermenting soybeans initial fungal growth i.e., *Rhizopus oligosporus*, for 24 hours followed by inoculation with *Micrococcus luteus* enhanced the concentration of isoflavones (diadzein, genistein and factor 2) concentration up to 69.9 %, whereas the amount of diadzein, genistein and factor 2 increased to 739,165 µg (75.74 %), 805, 855 µg (69.99 %) and 91, 542 µg (31.22 %) per gram of defatted soy bean powder, respectively after 48 h of *M. luteus* inoculation (Devanathi and Aditiwati, 2013). In another study Weber *et al*. (2013), demonstrated the extraction of genistein and daidzein from spent erythromycin fermentation. Defatted soybean meal is being sold as a protein and carbohydrate source in industrial scale fermentations for antibiotic productions. It is a reality that production scale fermentation requires almost 3 metric tonnes of soybean meal to prepare medium sufficient to a large fermenter, after fermentation and extraction of the erythromycin fermented broth will be discarded. Taking into account the isoflavone content of the soybean meal as around 0.1 – 0.2 % the total isoflavone content in the total erythromycin fermented broth amounts to around 3-6 kg. In this manner several fermenters operate on a regular basis in a single plant for antibiotic production. The researchers focussed on the extraction of isoflavones. It was noticed isoflavone aglycones are first converted to aglycones and further rhamnosylated by the erythromycin producing actinomycete, *Saccharopolyspora erythraea*. The genistein and daidzein, after extraction of erythromycin, were converted back to aglycon form either by acid hydrolysis or by enzymatic methods for the removal of rhamnose. In a final step the isoflavones were purified by solvent extraction, appearing to be an excellent example for production of value added utilization of materials which otherwise regarded as less valued waste streams in industrial production systems.

3.2. Red Rice

Red yeast rice is a part of the Chinese diet for several centuries and description about this product dates back to 800 AD during the Tang dynasty. The red rice is used in China to prepare rice wine and as food colourant and flavour. In the West, due to its medicinal properties it has led to the development of a product known as cholestin for use as a dietary supplement to reduce cholesterol levels in the body. A proprietary bioprocess is being used for the preparation of cholestin. The cholestin produced contains 0.4 % total HMG-CoA reductase inhibitors which is equal to 9.6 mg of HMC-CoA reductase inhibitor, however the recommended daily dose is 2.4 g cholestin, the rest 5.0 mg is reported to be lovastatin. Fermentation of rice with *Monascus purpureus* results in the production of Monacolin K or lovastatin, which reduces cholesterol biosynthesis in the body and the bioactive compound in the form of nutraceutical is available worldwide. The red rice along with Monacolin also contains substances that inhibit HMG-CoA reductase such as β-sitosterol, camesterol, stigmasterol and sapogenin, in addition to isoflavones and isoflavone glycosides and monounsaturated fatty acids. Weber *et al.* (2013) have shown that 204 g/day of red rice caused substantial lowering in LDL-cholesterol without affecting the HDL- cholesterol content in human trials. Cholestin was also found to reduce triglyceride levels due to the polyunsaturated fatty acid content of the product. The red rice is proposed as a real substitute for statin therapy which uses cholesterol lowering medication by reduction of HMG-CoH production in the liver (Fig 3). Side effects in the form of myalgia, myositis, or rhabdomyolysis are noticed in about 10-25% patients undergoing statin therapy, reduced on use of red instead of synthetic drugs (Chen *et al*., 2008).

3.3. Fermented Wheat Germ Extract

Fermented wheat is regarded as antioxidant rich and contains bioactive compounds in comparison to the non-fermented wheat. Fermented wheat germ extract (FWGE) involving the production of 2, 6-dimethoxy-p-benoquinone was invented by Mate Hidvegi, a Hungarian biochemist, in early 1990's. It is one of the food supplements in clinical application for cancer patients world over and used as a dietary supplement with a brand name Avemar®. FWGE is a mixture of bioactive compounds such as 2-methoxy benzoquinone and 2-dimethoxy benzoquinone largely responsible for its therapeutic properties. The patented bioprocess for the production FWGE comprise, preparation of extract from wheat germs and its fermentation by *Saccharomyces cerevisiae*, with continuous stirring for 18 hours, under controlled conditions of pH and temperature followed by separation of liquid portion and its filtration to separate cell mass. In subsequent steps the filtrate is vacuum concentrated and maltodextrins and colloidal silica dioxide were added to spray dry the mixture (Heimbach *et al*., 2007). It is also known that FWGE interacts synergistically with anticancer drugs and displays anti- metastasis in animal models in addition to immunomodulation down regulating MHC- complex and the induction of TNF-alpha. Avemar® was found to inhibit dissemination and proliferation of metastatic tumors before and after chemotherapy, surgery and radiation (Yeend *et al.*, 2012). It was also found to prevent chemical carcinogenesis and improve the status of some autoimmune diseases (Boros *et al*., 2005).

3.4. Fermented Animal Foods

Animal products such as fish, meat and milk including yoghurt, ham, salami, sausages and pastirma have been fermented from time immemorial by addition of salt and subsequent drying or fermentation. Fermentation of animal produce involves complex microbial ecosystems that combine bacteria, yeasts, and molds. Fermented meat products such are famous foods around the world, due to their characteristic texture, flavor and safety. The most important microorganisms responsible for product transformation during fermentation of animal by products are lactic acid bacteria (mainly *Lactobacillus* spp.) and coagulase-negative cocci (*Staphylococcus* and *Kocuria* spp). Many cured and fermented meat products are usually consumed raw with no need for further smoking or cooking. These products may consist of an entire meat piece which is ripened, dried or smoked or a mixture of minced meat and fat, which is stuffed into a casing, fermented, dried and/or smoked in form of sausages (Toldra, 2004). Salami (Europe), alheira (Portugal), androlla (Spain), nham (Thailand), kargyong, satchu, suka ko masu arjia, chartayshya and jamma (India and Nepal) and nem chua (Vietnam) are known for its health benefits (Prester, 2016).

4. Microbial Production of Nutraceuticals

4.1. Vitamin B_{12}

The dairy products enriched with added vitamins have attracted much interest and are referred as Food^{+} indicating their value addition and also renders health benefits beyond their normal nutritional value through increased amounts of bioactive compounds. Vitamins are essential in human nutrition and they are widely used as food supplements, vitamin enriched foods, and in cosmetics. "Nutra cells" is an European project in which *Propionibacterium freudenreichii* ssp *shemani* was used to fortify yoghurt with vitamin $B_{12,}$ using fermentation processes. It is known with names, cynocobalamin, it is water soluble, and is an essential vitamin in the human diet. The cobalamin includes cyanocobalamin, hydroxyl cobalamin, methylcobalamin, and 6'-deoxyadenosylcobalamin (Kalfitova and Sovjak, 2005). Humans cannot synthesize vitamin B_{12} and must come from microorganisms through fermented food sources or from consumption of meat. The vitamin B_{12} gained prominence during the 1960s and then faded gradually. The daily requirement of vitamin B_{12} for an adult is around 3 μg /day, its deficiency leads to pernicious anaemia. It is also reported that vitamin B_{12} is the most complex molecule found in living organisms. *Propionibacteria* are the well known producer of vitamin B_{12} in several fermentation processes involving milk and other substrates of plant and animal origin. A bacterial strain of *Propionibacterium freudenreichii* ssp *shermanii* was found to produce Vit B_{12}. Currently there are few bacteria that are reported to produce vitamin B_{12} viz. *Pseudomonas denitrificans*, *Bacillus megaterium* and *Propionibacterium*. Among these, *Propionibacterium* enjoys the GRAS status and the vitamin can be produced in food by fermentation process. Bacteria of the genera *Propionibacteria* are well known producers of vitamin B_{12}, in fermented foods (Fig 4). *Propionibacterium* could be grown in cheese whey as a cost-effective growth medium (Gardner and Champagne, 2005). In addition to the use of *Propionibacterium* for industrial production of vitamin B_{12}, the bacterium can be used to ferment dairy products to fortify the milk products with the vitamin. Consumption of fermented dairy product can replenish the requirement of vitamin B_{12} in the food chain. *Propionibacteria* are also used as starter cultures in Swiss-type cheeses (Hugenholtz and Smid, 2002).

4.2. Polysaccharides and Poly Amino Acids

Polysaccharides obtained from bacteria, fungi, yeast, plants and animals are important ingredients, among them, microbial polysaccharides have been recognized as nutraceuticals due to their health promoting properties. Bacteria produce several polysaccharides such as xanthan, gellan, curdlan, dextrans, cellulose and alginates and exopolysaccharides by lactic acid bacteria. Especially fungal polysaccharides i.e. scleroglucan, elsinan, lentinan demonstrate potential antitumor, antimicrobial, antioxidant hypercholesterolemic, and hypoglycaemic properties. Even the bacterial polysaccharide curdlan was found to elicit antitumor properties. Scleroglucan from *Sclerotium rolfsii* is widely reported to be an antitumor and antiviral polysaccharide. The animal polysaccharides such as hyaluronic acid chondritin and heparosan are currently produced by microbial fermentation instead of their extraction from sources. As a further advancement, *Escherichia coli, Lactococcus lactis*, and *Streptomycin albulus* fermentations have been developed in recent years. Bacteria are also potential producers of polyamino acids such as poly-gamma-glutamic acid, poly-epsilon-l-lysine and poly-l-ariginyl-poly(L-aspartic acid cyanophycin). Cyanobacteria produce cyanophycin in the presence of equimolar concentration of arginine and aspartic acid which can be used as starting material for preparation of dipeptides used for nutritional and therapeutic supplements. Epsilon poly-l-lysine, produced by *Streptomyces albulus* and other *Streptomyces* sp. is a polymer of l-lysine is non-toxic, associated with antibacterial and anticancer properties and is approved as food preservative in Japan and United States (Hiraki, 2003).

4.3. Conjugated linoleic Acid

Conjugated fatty acid such as linoleic acid (CLA) are found to reduce carcinogenesis, atherosclerosis and body fat and they attract much attention as nutraceuticals. Lactic acid bacteria were found to produce isomers of CLA from added linoleic acid. The CLA products comprise a mixture of cis-9, trans-11-octadecadienoic acid, 18:2 and trans-9, trans-11-18:2 (Ogawa et al 2005). *Lactobacillus plantarum* AKU1009a was selected as a potential CLA producer. Use of the washed cells of *L. plantarum* and incubation with linoleic acid resulted in the production of CLA up to 40μg/ml. The CLA biotransformation reaction was found to take place through two consecutive reactions i.e., hydration of linoleic acid to 10-hydroxy-12-octadecaenoic acid and dehydrating isomerisation of the hydroxyl fatty acid to CLA. Castor oil is rich in triacylglycerol form of ricinoleic acid to act as substrate for CLA production by lactic acid bacteria with the help of lipase catalysed triacylglycerol hydrolysis. *Lactobacillus plantarum* produces conjugated trienoic fatty acid from alpha and gamma-linolenic acid. The trienoic fatty acids were cis-9, trans-11, cis-15-18:3 octadecaenoic acid and trans-9, trans-11, cis-15-18:3 octadecaenoic acid. In another study linoleic acid obtained from corn oil using lipase from *Pseudomonas* sp was bio transformed into CLA or incorporated into butter oil by acidolysis and linoleic acid isomerases, obtained from rumen bacteria and *Candida antarctica* lipase fraction B.

4.4. Omega 3 fatty acids and polyunsaturated fatty acids

There Polyunsaturated fatty acids, particularly the omega 3 and omega 6 fatty acids i.e., eicosapentaenoic acid (EPA) 0: ω -6,12,15; gamma-linolenic acid (GLA) 18:3 ω 6, 9,12,15; docosahexanoic acid (DHA) 22:6 ω-3,6,9,12,15,18, and arachidonic acid (AA) 20:4 ω- 5,9,12,15 are in great demand due to their nutraceuticals benefits. These essential fatty acids are important to treat chronic inflammation conditions such as rheumatism, skin diseases, and hypercholesterolemia. The eukaryotic microalgae are known to contain higher amounts of polyunsaturated fatty acids compared to cyanobacteria. The main microalgae that are used for the production of PUFA are: *Nanochloropsis*, *Phaeodactylium* and *Porphyridiu*m in addition to, *Nitzschia*, *Spirulina*, *Isochrysis galbana* and *Cryptothecodium*. The content of PUFA production in these microalgae is dependent on growth conditions and growth stages. Increased PUFA production was shown to occur under nitrogen deficiency conditions, excess light as well as high salinity. Even *Haematococcus pluvialis* was shown to produce 15.6 % PUFA under optimal conditions and increase even up to 34.8 % when the cells are subjected to uninterrupted light and reduced nitrogen sources in the growth medium. *Schizochytrium limacium* and *Spirulina* were studied for PUFA production in saline waters. Thraustochytrids such as *Aurantichytrium* sp, *Schizochytrium* sp, *Thraustochytrium* sp and *Ulkenia*, and *Cryptothecodinium cohni* sp are well known synthesizers of >C20 long chain omega 3-oils and exopolysaccarides (Chang *et al.,* 2014). Ulkenia is a marine protist, non-parasitic, non-pathogenic and non-toxic. Large scale production of docosahexaenoic acid (DHA) is achieved using *Ulkenia* sp through a fermentation process. One of the essential ω 3- fatty acid for health promotion and it is mainly found in substantial quantities in brain and retina, and is essential for normal prenatal and infant neural development, DHA is also known to reduce cardiovascular problems in humans, and furthermore it was shown to reduce mental disorders such as Alzheimers's disease. The extracts obtained from microalgae such as *Chlorella* sp, *Chlorococcum humicola*, *Dunaliella* sp., *Porphyridium aerugineum* and *Chaetoceros lauderi* were also found to show antibacterial, antiviral and antifungal properties (Falaise *et al.,* 2016). The nutraceuticals obtained from marine sources comprise several algal products, bioactive peptides, protein hydrolysates, mucopolysaccharides, chitosan, and its hydrolysis products such as chito-oligosaccharides, and glucosamine. Both enzymatic and acid hydrolysis processes are being used to produce chitosan oligomers. Chitosan was found to be hypocholesterolemic, to cause weight loss, inhibitor of oxidation, and an agent for nutrient and control drug delivery, while oligomers of chitosan, N-acetylglucosamine and glucosamine have been shown to elicit anti-inflammatory and antimicrobial properties.

5. Valorization of Waste Products through Nutraceuticals Production

Less valuable materials resulting from rice milling food industry and other bioproccesing activities such as antibiotic productions were shown to yield important nutraceuticals using solid state fermentation processes. Rice bran, nutrient rich outer layer of the brown rice is discarded as waste by product has been used as animal feed. It is known to contain high quantity of protein, minerals, amino acids, lipase, and substantial quantities of antioxidants such as polyphenols including ferulic acid gamma oryzanols, tocopherols, and tocotrienoids (Li *et al.,* 2010). It also contains to a larger extent lipids, protein, vitamin B, and dietary fibre, moreover, it is known to inhibit melanogenesis (Chung *et al.,* 2009). Applications of rice bran for skin care and treatments are precluded because of its cytotoxicity to melanocytes. However, fermented rice bran extract showed inhibitory effect on melanogenesis by down regulating microphthalmia-associated transcription factor (MITF), a transcription factor responsible for the regulation of melanocyte- differentiation, pigment formation, proliferation and their maintenance. Through fermentation of rice bran, its cytoxicity to melanocytes was reduced, along with anti-allergic and anti-inflammatory properties, through inhibition of degranulation and hitamine release, and proinflammatory cytokine regulation. Rice bran is now being used as a source of important protein and fiber, for submerged and solid state fermentations to ferment food materials. According to the established methodology, very often solid state fermentation is applied to increase poly phenolic content of fermented substrate in comparison to submerged fermentation. Filamentous fungi such as *Aspergillus oryzae* and *A. awamori* are predominantly used for the purpose. *Cordyceps sinensis* is also found suitable for solid state fermentation of stale rice. In another study, phenolic compounds from fermented rice bran were found with enhanced anti-oxidant and antimicrobial properties (Scmidt *et al.,* 2014). Protein concentrate (69-70%) obtained from fermented rice bran contains essential amino acids lysine, threonine and valine to the extent of 6.93, 3.78, and 5.59 g/100g protein, respectively (Chinma *et al,* 2015). The fermented rice bran also contains anti-oxidant compounds, hence the protein concentrate extracted from fermented rice bran was found to be useful as a functional ingredient in the baking process. Furthermore, anti-fatigue effects of fermented rice bran produced using *Saccharomyces cerevisiae* are reported in animal models (Kim *et al*., 2001a). Chung *et al.* (2009) reported rice bran fermentation with *Lactobacillus rhamnosus* and *S. cerevisiae.* In addition the extract, fermentation was found to add several beneficial compounds by way of biotransformation of components already existed in rice bran.

6. Metabolic Engineering and Nutraceuticals Production

Metabolic engineering is a method to modify already existing metabolic path way or rearrangement or its complete construction in an organism to bring changes in biochemical pathways in a living organism with the help of gene insertion or deletion to increase low molecular weight metabolites or other products using recombinant DNA technology. In recent years low-calorie sugars have been produced using microorganisms as hosts (Patra *et al*., 2017). Polyols such as mannitol and sorbitol are the common low-calorie sugars that show the potential as substitutes for sucrose, lactose, glucose or fructose. Mannitol is also a known antioxidant in cell systems. *Leuconostoc mesenteroides* is known to produce mannitol from fructose. The bacterium uses a fraction of fructose energy production and another fraction is converted to energy, by metabolic engineering complete conversion of fructose to mannitol could be possible or with a new range of metabolites as was sown in case of *Lactobacillus plantarum* and *Lactococcus lactis*. It is another sweetener that has potential to replace sucrose as it has equal sweetness as sucrose or higher than mannitol, sorbitol and erythritol with much lower caloric value as it is poorly metabolizable in the human system. Tagatose has been launched into the market as low-calorie sugar, a prebiotic compound, as an anti-plaque compound. As of now the source of tagatose production enzymatic bioconversion of arabinose (Kim *et al.*, 2001b), however, whole microbial cells of acetic acid bacteria are used for the conversion galactitol to tagatose instead of a pure enzyme preparation.Using metabolic pathway engineering *Lactococcus lactis* was made to produce L-alanine from sugar. For this the gene for alanine dehydrogenase (alaD) was cloned from *Bacillus sphaericus* into *Lactococcus lactis* lacking lactate dehydrogenase system (Manzoni *et al.*, 2001). The alanine dehydrogenase was intended to convert pyruvate to L-alanine using ammonium as another substrate. Alanine is an amino acid with sweetness equal to glucose or fructose. Production of low-caloric sugars such as mannitol, sorbitol, tagtose and alanine have been touched upon here as they are known to be nutraceuticals in their physiology with health promoting benefits (Hugenholtz *et al.*, 2002).

7. Conclusion

Nutraceuticals are endowed with nutritive, prophylactic or therapeutic properties to fight chronic diseases. Plants, animals and microorganisms are sources of a large variety of nutraceuticals. They are found to be anticancer, antioxidant, anti-diabetic, anti-atherogenic, immunomodulatory, osteosynthetic, anti-fatigue and anxiolytic and improve the gastrointestinal health synergistically along with probiotics and prebiotics. They are a large number of probiotic microorganisms particularly that belong to the genera *Lactobacillus* and *Bifidobacterium*. Prebiotics such as fructo-oligosaccharides, malto-oligosaccharides and inulin can enhance the growth and metabolic activity of probiotics. Fermentation of plant and animal foods could lead to the enrichment with vitamins, fatty acids and several bioactive compounds for absorption in the body. Waste materials or less-valued food processing byproducts can be efficiently used for enhancing the nutritional quality and to enrich with nutraceuticals. It was shown that enzymatic bio transformations and lactic acid bacteria have potential to produce conjugated linoleic acid. The importance of fermentation has been dealt to a reasonable detail. Finally, it is

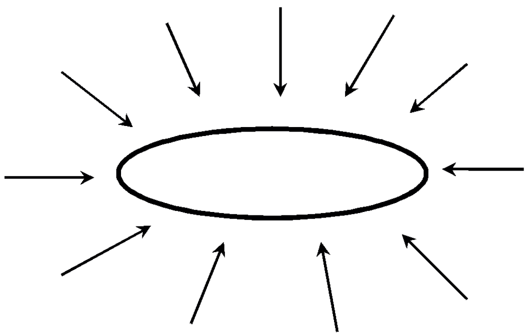

Fig. 1: The multidisciplinary nature of nutraceuticals production and its application

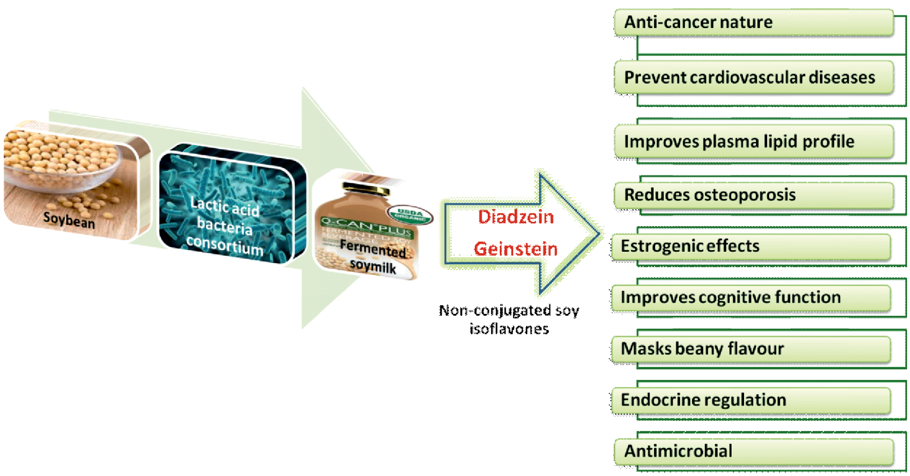

Fig. 2: Fermented soy milk and its benefits

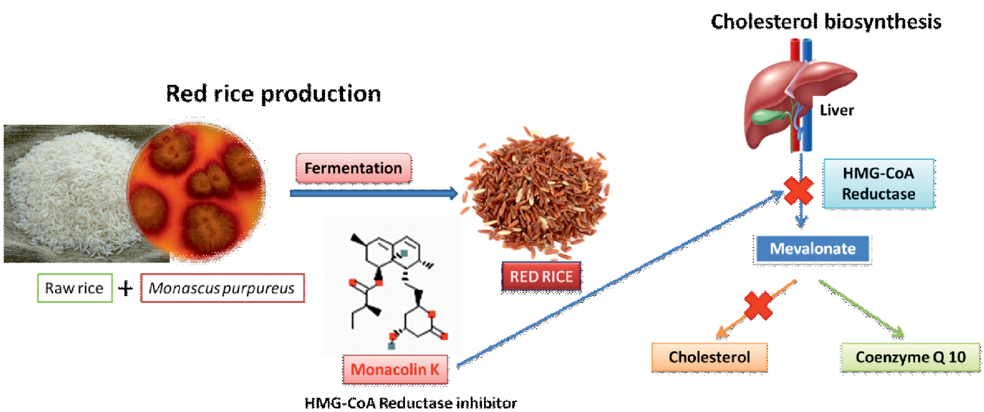

Fig. 3: Effect of red rice consumption on cholesterol biosynthesis

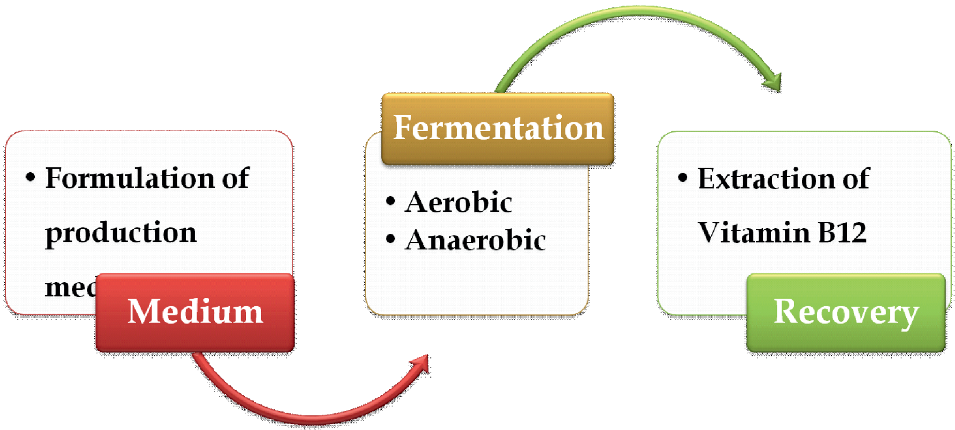

Fig. 4: Microbial production of vitamin B_{12}

worthwhile to reiterate that nutraceuticals is a vast field and multidisciplinary arena which needs strong analytical and downstream processing expertise. Modern enzymatic extraction methodologies which are greener to the environment have to be harnessed for obtaining potential nutraceuticals to satiate food and nutritional requirements and demand of burgeoning world population. Several types of less valued materials resulting from food processing activity can be converted into nutrient rich and nutraceuticals containing food products using pretreatment and fermentation processes, thus providing opportunity to fulfill the demand for food. Further studies are required in this direction to achieve these goals.

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