CHAPTER 2

Biotechnology in Food Processing and Preservation: An Overview

Gargi Ghoshal

Dr. S.S. Bhatnagar University Institute of Chemical Engineering & Technology, Panjab University, Chandigarh, India

1 Introduction

To fabricate and develop commercial products and methods by employing molecular methods that utilize entire or fractions of living organisms is known as modern biotechnology. Modern biotechnology is relatively recent and quick growing division of the molecular biology that was introduced 30 years ago with the development of the first recombinant gene. Biotechnology is changing our way of living by affecting the foods, drinks, medicine, and cloths. The application of biotechnological methods in the food and agricultural industry has great repercussion on the society. Biotechnology has the maximum potential to resolve the pressing need of hunger today and thus help to avoid mass starvation in the coming future.

Through domestication and agricultural activities of breeding and selection, plants were developed into food crops that permit fabrication of more healthy, safer, tastier, and nutritious edible item. Various other aspects of biotechnology, such as medical biotechnology, also known as red biotechnology, help us in gene therapy, initial stage identification of various diseases, such as cancer, diabetes, Parkinson's, Alzheimer's, and atherosclerosis resulting in early stage treatment and eventually curing these diseases.

Palatable foods and potable beverages can be manufactured by converting relatively huge amount of perishable and nonedible food materials into more useful and shelf stable products by using various unit operations and technologies. Any kind of technology applied in the food processing must be safe and good quality and the final product must be free from any health hazard. Definition of safe food is the food, which is chemically, physically, and microbiologically free from any harmful material; or the level of contaminant present in the food will not cause any harm to public health. Nowadays, consumers are interested to pay the premium for quality food products that are safe and convenient.

A range of technologies is applied at different levels and scale of operation in food processing across the developing country. Low input and conventional technology includes drying,

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evaporation, canning, dehydration, freezing, vacuum packing, osmotic dehydration, sugar crystallization, etc.

Processing assures food security by minimizing waste production and reducing the food chain and increasing food availability and marketability. The purpose of food processing is to improve its quality and security. Food safety is a scientific discipline, which ensures that a particular food will not be the reason of any injury to the consumer when it is manufactured and eaten according to its deliberate use. Biotechnology plays a pivotal role to improve the taste, flavors, color, texture, aroma of foods, and its aesthetic and nutritional value; it is extensively used in many countries. Food undergoes fermentation by intentional inoculation or by natural fermentation and eventually these desirable changes appear due to fermentation by microorganisms and/or their enzymes, flavor, fragrance, food additives, and other value-added products. These high value products are used in food and nonfood use and also imported to other countries.

Food processing involves various unit operations and techniques to convert raw, perishable, and inedible products to consumable form with enhanced quality and shelf life. To produce a safe and high quality food, the process and manufacturing protocol used in the food processing must be of food grade, that is, free from health hazards. Safe food can be defined as the food that contains no harmful components that affects human health and nutrition. Biotechnology is also widely employed as a tool in diagnostics to monitor food safety, prevent, and diagnose food-borne illnesses and verify the origin of foods. Techniques applied in the assurance of food safety focus on the detection and monitoring of hazards whether biological, chemical, or physical. Fermentation is generally used to make desirable changes in food. Fermentation can be carried out naturally or by intentional inoculation. Fermentation is the process in which carbohydrates are converted into alcohol and carbon dioxide or organic acids when yeasts, bacteria, or a combination of them works on the food in the absence of air. Fermentation is used to produce wine, beer, cider, leavening of bread, and lactic acid.

Ghoshal (2012) studied the effect of xylanase from *Penicillium citrinum* on rheological properties of whole-wheat dough. Linear viscoelastic range was observed from 0.1% to 1%. The amplitude sweep test established that G′ and G″ were higher in xylanase containing dough as compared to control. The values of power law coefficient, x and y were higher in xylanase containing dough, which showed higher dependency on strain. In weak gel model parameters, higher A value revealed the stronger starch gluten network in xylanase containing dough, while lower value of z represented the higher dependency of both the modulus (G′ and G″) on the strain. Creep compliance data revealed that control dough is stronger than xylanase containing dough. Creep test data of control and xylanase containing dough were fitted to Peleg, Kelvin, and Burger Models to check the adequacy of fitting of creep data in to different mathematical models. Peleg model, as well as six-element Kelvin model described well the creep behavior of control and xylanase containing dough samples. Large deformation of dough in terms of uniaxial extensibility and unfermented dough stickiness study revealed that

xylanase containing bread exhibited greater extensibility and less resistance to extension as compared to control samples. It is found that R_m (maximum resistance) was higher in control than enzyme treated dough but extensibility (E) increased with enzyme supplementation. Therefore, it was concluded that xylanase addition makes the dough softer. SEM study revealed that addition of xylanase resulted in continuous and closed gluten network in which starch granules are embedded. Higher magnification revealed that large starch granules were more swollen and evenly dispersed within the protein matrix.

Ghoshal et al. (2013) studied the effect of xylanase on whole wheat bread. The quality of bread containing xylanase was improved with respect to specific volume and moisture loss, textural properties, color, thermal properties, and sensory properties. Firmness values and enthalpy values of stored samples were fitted in Avrami equation. Xylanase addition resulted in the reduced rate of staling in bread. A 20% reduction of limiting firmness value was observed in bread containing xylanase. Using Avrami equation, calculated values of firmness and enthalpy were determined and plotted. The calculated values were in agreement with the experimental values. During storage, bread-containing xylanase was softer as compared to control. From the analysis of various staling properties examined, it can be inferred that bread stales at both ambient (25°C) and cold (4°C) temperature, but the rate was lowest at cold (4°C) temperature in bread containing xylanase. From the aforementioned study, it was proved that partially purified xylanase could be used to improve the color, texture, and sensory properties of whole wheat bread.

Kaur and Ghoshal (2016) studied the biocolor production using selected fruits and vegetable peel and orange red color was extracted using the strain *Blakeslea trispora* (+) MTCC 884 by solid-state fermentation. Analytical determination of color using UV-spectrophotometer produced maximum absorbance at 449 nm which confirmed that extracted color was β-carotene and the peak of HPLC analysis curves at retention time of 12–14 min further confirmed the chromatograms of β-carotene. It has been observed that β-carotene production was influenced by the parameters, such as pH, temperature, and incubation time. It was also concluded that 96 h at 28°C and pH 6.2 were the most appropriate environmental parameters for the production of β-carotene. Mass spectroscopy of extracted color displayed the m/z value at 537.608 agreeing to the presence of β-carotene. LCMS analysis of extracted color gave the eluted peaks of trans β-carotene (Rt 13.37) confirmed the presence of β-carotene.

Natural fermentation leads human history. The earliest evidence of fermentation dates back to 7000–6000 BC. It was an alcoholic beverage, made from fruits, rice, and honey in the Neolithic age in Chinese village of Jiahu. Winemaking was prevalent in 6000 BC in Georgia. There was a jar containing traces of 7000 years old wine displayed at the University of Pennsylvania, excavated from mountains in Iran. Also the traces provided the proofs regarding the production of fermented products in Babylon c.3000 BC, ancient Egypt c.3150 BC, pre-Hispanic Mexico c.2000 BC, and Sudan c.1500 BC.

Louis Pasteur, French chemist was first to connect yeast to fermentation in 1856. He defined fermentation as respiration without air. Fermentation, useful for conversion of sugars and other carbohydrates into preservatives and other organic acids, is the result of his research. Fermentation is generally used in food processing as it:

- modifies diet by enrichment of flavors, aromas, and food texture;
- preserves food by production of acids;
- enriches food with protein, essential amino acids, and vitamins;
- removes antinutritional factors; and
- decreases process time.

This review is regarding the recent advances in biotechnology and its applications in food processing and manufacturing of various foods from transgenic plants, animals, and microorganisms. Plants are the primary source of food for humans and feed for livestock. Through domestication and agricultural activities of breeding and selection, plants were developed into food crops that serve as the major source of dietary carbohydrates, lipids, proteins, vitamins, and minerals for humans and livestock. This part of the article discusses the occurrence of genetic engineering to improve the quality of milk in cattle, reduce the fat content in swine, increase the growth and productivity in poultry, and provide tolerance against freezing temperatures in fish. The fabrication of a variety of proteins by using mammary glands and eggs as bioreactors and modification of microorganisms by genetic engineering for improvement of food products has also been discussed. Various biotechnological techniques for the identification of transgenic substances and harmful pathogens are also described.

1.1 Methods to Improve the Quality of Microbial Strain

In traditional biotechnology, microbial cultures are improved for use in food processing application by improving the quality of microorganisms and the yield of metabolites using mutagenesis, conjugation, and hybridization (for yeast *Saccharomyces cerevisia* strain used in baking, brewing, and beverage production methods).

Recombinant genetic engineering is the best-known technique to alter the purified microbial strain related to food fermentations following the norms and regulations as per customer awareness. Genetically modified (GM) strains are applied in the manufacture of enzymes, vitamins, PUFA, amino acids and other fatty acids (Tables 2.1 and 2.2).

2 Genetically Modified Plants

2.1 Methods of Production of Genetically Modified Plant

GM modified plants are generated by the biolistic method (Particle gun method) or by *Agrobacterium tumefactions* mediated transformation method. In biolistic or gene gun

Table 2.1: Applications of some food additives and processing aids derived from Genetically modified (GM) microorganisms.

Applications	Categories of Food Additives	Food Additives
Cheese making	Enzymes	Rennet
Manufacturing of high fructose corn syrup		Isomerase, amylase
"Lite" beer		Pullulanase
Meat tenderizer		Proteases
Juice, beer clarification, and bread manufacturing		Xylanase
Nutritional supplement	Amino acids	Methionine, lysine, and tryptophan
Ingredient in sweetener production		Aspartic acid and phenylalanine
Acidulant	Organic acids	Citric acid, acetic acid, benzoic, and probionic acid
Flavoring and coloring agents	Flavors and pigments	Vanillin and monascin
Nonnutritive sweeteners	Low-calorie products	Aspartame, thaumatin, and monellin
Food additives, and cooking oil		Modified fatty acids triglycerides
Animal and human food supplement	Single-cell protein	
Stabilizers, thickeners, and gelling agents	Microbial polysaccharides	Xanthan gum

Table 2.2: Enzymes from GM microorganisms.

Enzymes	Source Microorganisms
Chymosin	*Aspergillus niger*
Phytase	
Lipase	
Aspartic proteinase	*Aspergillus oryzae*
Esterase-lipase	
Glucose oxidase	
Laccase	
Lipase	
α-Amylase	*Bacillus licheniformis*
Pullulanase	
α-Acetolactate decarboxylase	*Bacillus subtilis*
α-Amylase	
Maltogenic amlyase	
Pullulanase	
Chymosin	*Escherichia coli* K-12
Xylanase	*Fusarium venenatum*
Chymosin	*Kluyveromyces marxianus* var. *lactis*
α-Amylase	*Pseudomonas fluorescens*
Pectin lyase	*Trichoderma reesei*

method, the gene is directly shot at the plant cell under high pressure. This method has been applied successfully for many crops, especially monocots, such as wheat or maize, for which transformation using *A. tumefaction's* has been less successful. This technique is clean and safe. The only disadvantage of this process is that serious damage can happen to the cellular tissue. In agrobacterium-mediated piece of DNA, which infects a plant is integrated into a plant chromosome, through a tumor inducing plasmid along with the genetically engineered (GE) strain.

Recent advancements in plant sciences and agricultural biotechnology offer new opportunities and possibilities to improve the yield, quality, and production economics of food crops. Few examples are given to generate vitamin, mineral, and essential nutrient-rich transgenic plants (Klein et al., 1987).

2.2 Vitamin-Rich Plants

Vitamins play a vital task on human health by varying metabolic circumstances and supporting the biochemical processes that liberate energy from foods during digestion, making of hormones, blood cells, nervous-system chemicals, and other genetic materials. Deficiency of any vitamin can cause serious health disorder. Transgenic plants can be manufactured using knowledge of biotechnology, with increased content of vitamins in certain crops.

2.2.1 Vitamin-A

Reduced form of vitamin A (retinal) is the source of rhodopsin. Rhodopsin is essential for vision and also employs to preserve epithelial and immune cells. The retinoic acid is essential during the embryonic development and for homeostasis in adult body and its scarcity develops the sign of night blindness to total blindness. Fruits and vegetables are principal source of β-carotene and are predecessors of vitamin-A (Fig. 2.1). In carotenoid pathway, the extent of β-carotene formed by plants can be enhanced by increasing the flux by raising the availability of carotenoid precursors, by communicating enzymes in the early part of the pathway between geranyl pyrophosphate and lycopene (Fig. 2.2).

For example, transgenic rice was developed by Swiss Federal Institute of Technology, Zurich, Switzerland in collaboration with University of Freiburg. In this transgenic rice, expressing genes for β-carotene was incorporated (Potrykus, 2001). Four steps are involved in β-carotene biosynthesis of rice grain (Bartley et al., 1994). This rice appeared yellow in color. β-Carotene (1.6 μg) is present in selected line per gram of rice endosperm, and was recognized as "golden rice." The first strategy is production of carotenoid over expression in tomato by using DXP synthase that enhances flux in the entire pathway and enhances the total carotenoid content (Enfissi et al., 2005).

Cho et al. (2016) studied the comparison of nutritional characteristics of transgenic rice containing CaMsrB2 gene and traditional variety. This study was conducted to compare

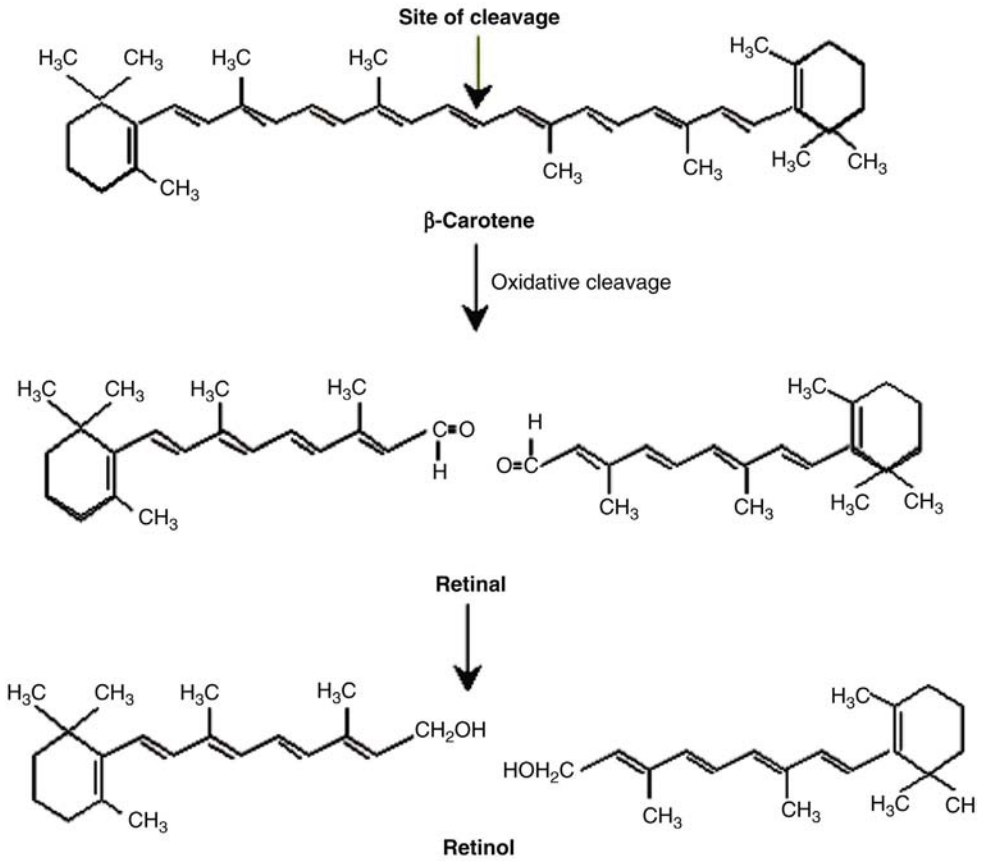

Figure 2.1: Biosynthesis of Vitamin A.

nutritional profiles of compositional analysis in terms of proximate components, lipid profiles, amino acid profile, and vitamin contents, and antinutrients between transgenic drought-tolerant Agb0103 rice harboring the pepper methionine sulfoxide reductase B2 gene CaMsrB2 and the parental rice cultivar, "Ilmi" as a nontransgenic control. And found that Agb0103 rice with improved resistance to drought is nutritionally equivalent to the parental rice cultivar.

2.2.2 Vitamin-C

Ascorbic acid is an important antioxidant and cofactor for various enzymes. It improves immunity, boost cardiovascular functions, alleviate ailment relating to connective tissue (Davey et al., 2000), and it is essential for iron metabolism (Hallberg et al., 1989). Humans cannot synthesize ascorbic acid due to absence of L-gulono-1,4-lactone oxidoreductase, which is needed during biosynthesis of ascorbic acid. Vitamin-C-rich plants are the only dietary sources of vitamin-C for humans (Davey et al., 2000). In plants, biosynthesis of

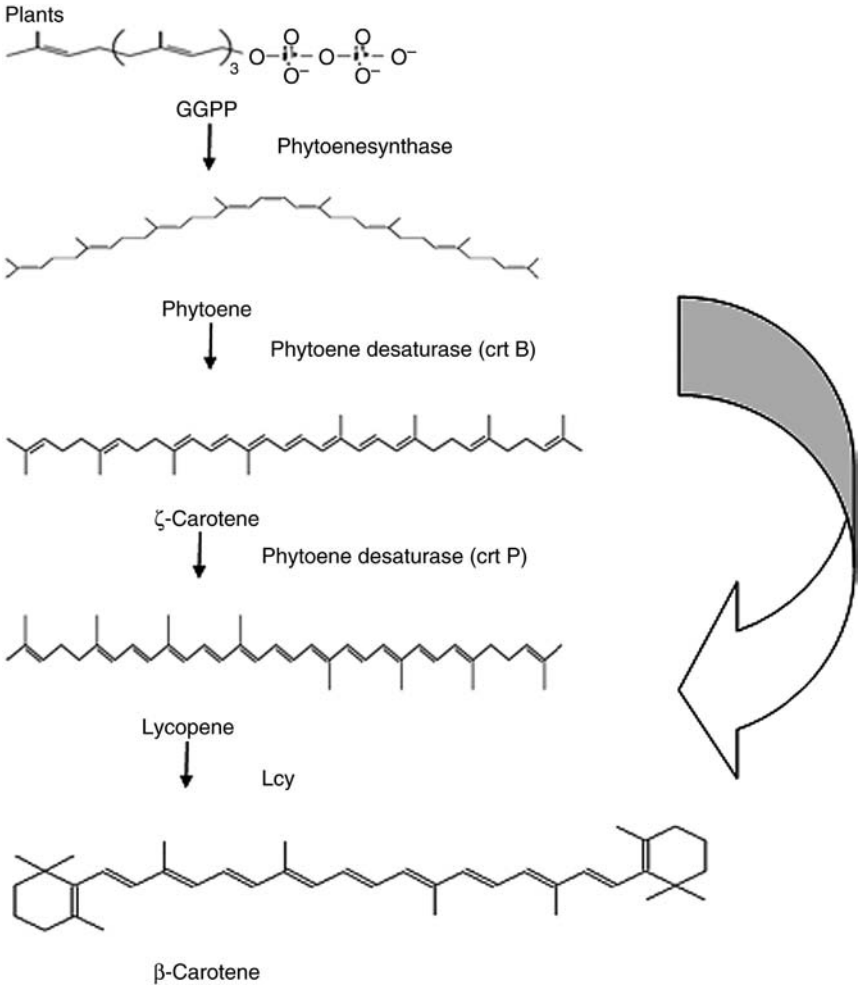

Figure 2.2: Conversion of β-Carotene From Geranyl Geranyl Pyrophosphate (GGPP).
Lcy, Lycopene cyclase.

vitamin-C takes place in two ways. First, with the conversion of D-galactouronic acid to L-galactouronic acid by D-galactouronic acid reductase enzyme followed by conversion of L-galactouronic acid to L-galactano-1,4-lactone, immediate predecessor of ascorbic acid. Agius et al. (2003) isolated D-galacturonic acid reductase enzyme encoding gene from strawberry (Fig. 2.3) (Smirnoff et al., 2001; Wheeler et al., 1998) and characterized as *galUR*.

In alternative method vitamin-C is synthesized by recycling (Smirnoff et al., 2001; Washko et al., 1992; Wheeler et al., 1998). Chen et al. (2003) hypothesized that by enhancing the expression of DHAR in plants, ascorbic acid synthesis also increases, and a proficient ascorbate recovery would be accomplished. Following the ascorbate recycling pathway,

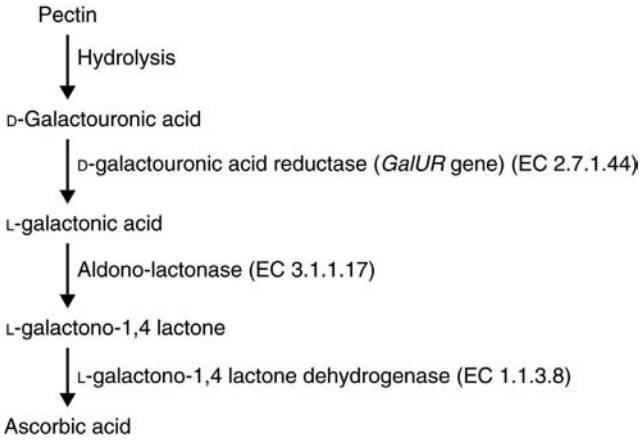

Figure 2.3: Biosynthesis of Vitamin C.

expressing the rice *dhar* gene in multivitamin maize, 6 times enhanced level of ascorbate compared to normal level had been observed (Fig. 2.4).

2.2.3 Vitamin-E

Vitamin E belongs to tocotrienol and tocopherol families and is lipid soluble. Mainly in plants, vitamin is produced during photosynthesis (Hess, 1993). Vitamin-E is important because of its therapeutic properties. It is best known for its activity against cancer, degenerative disorders, and cholesterol (Theriault et al., 1999). Tocotrienol is more powerful antioxidant than tocopherol but not absorbed as readily. Researchers have taken initiative to grow vitamin-E-rich plants. Various methods are being used.

Vitamin-E is not a single vitamin; actually it describes eight fat-soluble antioxidants from the tocotrienol and tocopherol families that are synthesized by plants photosynthetic pathway

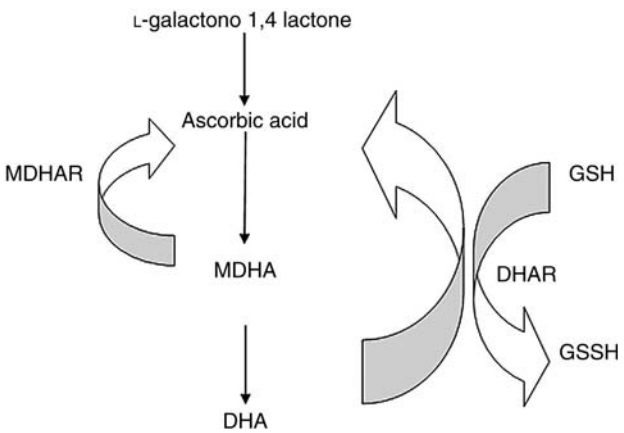

Figure 2.4: Recycling of Ascorbic Acid.

Table 2.3: Essential minerals for human being.

Macronutrients	Micronutrients
Ca, P, Na, Mg, Cl, S, Si	Fe, F, Zn, Cu, Co, I, Se, Mn, Mb, Cr

(Hess, 1993). During biosynthesis of both tocopherols and tocotrienols, homogentisic acid formation from *p*-hydroxyphenyl-pyruvate is the first step and it is catalyzed by the enzyme *p*-hydroxyphenyl-pyruvate dioxygenase (EC 1.13.11.27) (Grusack and DellaPenna, 1999). In another method, identification and isolation of a novel monocot gene that encodes HGGT; (specific enzyme for tocotrienol synthesis) is done for enhancement of vitamin E (Cahoon et al., 2003). A 10–15-fold increase in tocotrienol synthesis is noticed in bioengineered barley with HGGT.

The third way involves manipulation of final step in biosynthesis of vitamin-E to enhance the vitamin-E content. Hereby using the enzyme γ-tocopherol methyl transferase as catalyst the conversion of γ-tocotrienol and γ-tocopherol to α-tocotrienol and α-tocopherol is taken place (Shintani and DellaPenna, 1998).

2.3 Essential Minerals

Essential mineral content (micro and macronutrients) are listed in Table 2.3.

2.3.1 Iron

Iron insufficiency is the most common mineral malnutrition worldwide; more than 2 billion people suffer from iron deficiency along with primary clinical symptom of anemia about half of iron deficiency cases. To avoid this, several techniques, such as enrichment of food with iron and other functional ingredients are being exploited (Maberly et al., 1994), but the success is limited, especially in developing nations. So instead of supplementation, the new techniques of bioengineering are used to enhance the essential mineral content in staple food crops. Strategies implied are over expression of ferreting, store large quantity of bio available iron, and the expression of photoset, which degrade phytate, which inhibit many essential mineral, (Ravindran et al., 1995) and help to absorb easily stored iron in the human digestive system. Goto et al. (1999) introduced soybean ferritin cDNA into rice plants, which endorsed the accretion of iron in rice grain endosperm 3 times more than the untransformed plants. Lucca et al. (2002) inserted fungal (*Aspergillus niger*) phytase cDNA in rice and increased degradation of phytic acid was observed.

2.4 Essential Amino Acids

Human beings cannot synthesize essential amino acids on their own. Out of 20 amino acids, nine amino acids histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine,

tryptophan, and valine are essential amino acids. Animal proteins are the complete sources of amino acids. Plant sources, such as cereals are deficient in lysine and threonine, legumes are deficient in tryptophan, methionine, and/or cysteine. To make more nutritious plant sources, it is essential to incorporate amino acid-rich gene and to make GE plant. Amino acid deficiency can be tackled by applying two GE approaches: (1) introducing engineering plants to generate proteins including essential amino acids; and (2) incorporating engineering design in amino acid metabolism to enhance the accessibility of essential amino acids in the free amino acid basket. Lysine was chosen first in both the approaches. As lysine deficiency results in fatigue, lack of concentration, bad temper, pale eyes, delayed growth, hair loss, anemia, and reproductive problems. Zheng et al. (1995) had taken initiative to introduce β-phaseolin, a gene from the common bean (*Phaseolus vulgaris*) to fabricate transgenic rice with improved lysine content. To transmit gene protoplast, mediated alteration process was used. Transgenic maize seeds express the AH protein contained up to 32% more protein than wild-type seeds and contained higher levels of lysine, tryptophan, and isoleucine. To produce lysine, methionine, and tyrosine-rich transgenic potato, *Agrobacterrium* mediated transformation was done.

2.5 Essential Phytochemicals

Phytochemicals are precious for human nutrition. Indoles, isothiocyanates, and sulforaphane from vegetables, such as broccoli, alylic sulfides from onions and garlic and isoflavonoids from soybeans are known as plant phytochemicals. These are present in high concentration in raw foods but intensities are reduced during processing and handling (Wang and Murphy, 1996). Enhanced amount of phytochemicals in foods can resolve this difficulty. Two genes IFS1/IFS2, encoding for isoflavone synthase in soybean are revealed and expressed in *Arabidopsis thaliana*, to activate the synthesis of isoflavonoid genistin (Jung et al., 2000).

2.6 Isoflavonoids

Flavonoids are a group of phytochemicals responsible for the pigmentation in plant, feed deterrence, wood protection, protection from fungi and insects, and introduction of genes for root nodulation. Anthocyanins, condensed tannins, and isoflavonoids, are flavonoids, actually they are phytochemicals (Buchanan et al., 2001). The major resources of isoflavonoids can be achieved by consumption of soybean-rich products. Isoflavonoid levels can decrease by 50% during soya seed processing for traditional soy foods (Wang and Murphy, 1996). Escalating isoflavonoid quantity in soybean could solve this problem. Alternatively, development of other isoflavonoid-rich crops that can create this powerful compound therefore, widens their consumption. Jung et al. (2000) recognized *IFS1/IFS2*, two soybean genes encoding isoflavone synthase, and expressed these genes in *A. thaliana*, generating the synthesis of the isoflavonoid genistein. Approximately 2 ng/μg of fresh plant weight, genistein was produced (Jung et al., 2000).

Table 2.4: Fermented foods of different countries.

S. No.	Fermented Foods	Countries of Origin
1	Amazake, atchara, bai-ming, belacan, burong mangga, com ruou, dalok, doenjang, douchi, jeruk, lambanog, kimchi, kombucha, leppet-so, narezushi, miang, miso, nata de coco, nata de pina, natto, sake, seokbakji, soju, soy sauce, stinky tofu, szechwan cabbage, tai-tan tsoi, chiraki, tape, tempeh, and totkal kimchi	East and Southeast Asia
2	Kumis (mare milk), kefir, and shubat (camel milk)	Central Asia
3	Achar, appam, dosa, dhokla, dahi (yogurt), idli, kaanji, mixed pickle, ngari, hawaichaar, jaand (rice beer), sinki, tongba, and paneer	India
4	Fermented millet porridge, garri, hibiscus seed, hot pepper sauce, injera, lamoun makbouss, laxoox, mauoloh, msir, mslalla, oilseed, ogi, ogili, ogiri, and iru	Africa
5	Sourdough bread, cultured milk, chicha, elderberry wine, kombucha, pickling (pickled vegetables), sauerkraut, lupin seed, oilseed, chocolate, vanilla, tabasco, tibicos, pulque, and mikyuk (fermented bowhead whale)	United States
6	Kushuk, lamoun makbouss, mekhalel, torshi, boza	Middle East
7	Rakfisk, sauerkraut, pickled cucumber, surströmming, mead, elderberry wine, salami, sucuk, prosciutto, cultured milk products, such as quark, kefir, filmjölk, crème fraîche, smetana, skyr, rakı, and tupí	Europe
8	Poi, kaanga pirau (rotten corn), and sago	Oceania
9	Idli, dosa, dhokla, jellabi, kefir, and kam	India

2.7 Enzymes

Enzymes occur in all living organisms and catalyze biochemical reactions that are necessary to support life (Olempska-Beer, 2008). They are commonly used in food processing, preservation, and raw ingredient manufacturing. The use of recombinant DNA technology has made it possible to manufacture novel enzymes that are tailored to specific food processing conditions. Alpha amylases with increased heat stability have been engineered for use in the production of high-fructose corn syrups. These improvements were accomplished by introducing changes in the α-amylase amino acid sequences through DNA sequence modifications of the α-amylase genes (Olempska-Beer, 2008). Enzymes derived from recombinant microorganisms are listed in Table 2.2 and other application of enzymes in food processing is listed in Tables 2.4–2.6. Application of enzymes in food preservation and manufacturing has historically been considered nontoxic.

2.8 Flavors, Amino Acids, and Sweeteners

Volatile organic chemicals, such as flavors and aromas are the sensory principles of many consumer products and govern their acceptance and market success (Berger, 2009). Flavors produced using microorganisms currently compete with those from traditional agricultural sources. According to Berger (2009), more than 100 commercial aroma chemicals are derived

Table 2.5: Categorization of different fermented foods on the basis of raw materials.

S. No.	Raw Materials Used	Names of Products
1	Cereal-based (with/without pulses) fermented foods	Amazake, beer, bread, choujiu, gamju, injera, kvass, makgeolli, murri, ogi, rejuvelac, sake, sikhye, sourdough, sowans, rice wine, malt whisky, grain whisky, idli, dosa, vodka, and boza
2	Milk-based fermented foods	Some kinds of cheese also, kefir, kumis (mare milk), shubat (camel milk), cultured milk products, such as quark, filmjölk, crème fraîche, smetana, skyr, and yogurt
3	Vegetable, BS, and unripe fruits-based fermented foods	Kimchi, mixed pickle, sauerkraut, Indian pickle, gundruk, and tursu
4	Pulse (legume)-based fermented foods	Cheonggukjang, doenjang, miso, natto, soy sauce, stinky tofu, tempeh, oncom, soybean paste, Beijing mung bean milk, kinama, and iru
5	Honey-based	Mead and metheglin
6	Tea-based	Pu-erh tea and kombucha
7	Fish-based	Bagoong, faseekh, fish sauce, Garum, Hákarl, jeotgal, rakfisk, shrimp paste, surströmming, and shidal
8	Meat-based fermented foods	Chorizo, salami, sucuk, pepperoni, nem chua, som moo, and saucisson

BS, Bamboo shoot.

using biotechnology either through the screening for overproducers, the elucidation of metabolic pathways and precursors or through the application of conventional bioengineering. Recombinant DNA technologies have also enhanced efficiency in the production of nonnutritive sweeteners, such as aspartame and thaumatin. Market development has been particularly dynamic for the flavor enhancer glutamate (Leuchtenberger et al., 2005), which is produced by the fermentation of sugar sources, such as molasses, sucrose, or glucose using high-performance strains of *Corynebacterium glutamicum* and *Escherichia coli* (Table 2.7).

Balsamo et al. (2016) studied proteome comparison of grains from two maize genotypes, with colorless kernel pericarp, P1-ww and red kernel pericarp, P1-rr. Two-dimensional gel electrophoresis (2-DE) was performed from univariate analysis identified three soluble protein extracts of each maize genotype and 55 proteins spots. Multivariate analysis showed the separation of the two maize genotypes proteome profiles using 2-DE data.

Enzyme plays a vital role in oil extraction, purification, and modification. Microbial lipase is extensively used in oil extraction, purification, and oil modification. Commercial Cocoa Butter Equivalent may be produced by combination of different processing steps, including blending, interesterification, fractionation, and refining using lipase (Fig. 2.5).

2.9 DNA Vaccine

GE DNA is introduced directly into the body to defend an animal against any disease where cell can produce an antigen, resulting in a protective immunological response. There has

Table 2.6: Production of enzymes using different microorganisms and substrates.

Enzymes	Microorganisms	Substrates
Cellulase, β-glycosidase, CMCase, laccase, xylanase, polygalactouronase, ligninase	Strains of *Aspergillus* sp., *Trichoderma* sp., *Lentinula* sp., *Penicillium* sp., *Pleurotus* sp., *Sporotrichum* sp., *pulverulentum*, *Cerrena* sp., *Bortritis* sp., *Gliocladium* sp., *Phanerochaete* sp., etc.	Bagasse, coconut coir pith, rice husk, rice straw, wheat bran, wheat straw, tea waste, sweet sorghum, silage, sugar beet pulp, saw-dust, grape-wine cutting waste, palm oil mill waste, sago hampas, cassava waste, sweet sorghum, soy hull, paddy straw, etc.
Xylanases, β-xylosidase, α-arabinofuranosidase, acetoesterase, catechol-oxidase	Strains of *Aspergillus* sp., *Trichoderma* sp., *Penicillium* sp., *Phlebia radiate*, *P. eryngii*, *Melanocarpus albomyces*, *P. sanguineous*, *Thermomyces lanuginose*, *Thermascus aurantiacus*, *Talaromyces emersonii*, *Thermomono spora* sp.	Rice straw, corn hull, corncobs, wheat bran, wheat straw, bagasse, rice straw, cotton stalks, soy hull, kraft pulp, sugar beet pulp, rice husk, apple pomace, corn cobs, coffee processing waste, barley straw, and oat straw
Laccase, Li-peroxidase, Mn-peroxidase, aryl alcohol oxidase, catalase, phenol oxidase	Strains of *Penicillium* sp., *Pleurotus* sp., *Phlebia radiate*, *Trametes versicolor*, *Flammulina velutipse*, *Polyporus* sp., *Panus tigrinus*, *Trichoderma versicolor*	Bagasse, wheat bran, wheat straw, sawdust, cotton stalk, kraft lignin, cellulose powder, and wood chips
Protease (acidic, neutral, and alkaline)	Strains of *Aspergillus* sp., *Penicillium* sp., *Rhizopus* sp., *Bacillus* sp., *Trichoderma* sp.	Wheat bran, sunflower flour, coffee husk, soybean meal, rice bran, corn bran, rice hull, aspen wood, sweet potato residue, and waste hair
Lipase	Strain of candida sp., *Aspergillus* sp., *Rhizopus* sp., *Neurospora sitophila*, *P. candidam*, *Mucor* sp.	Wheat bran, peanut cake, and rice bran
α-Galactosidase, β-galactosidase	*A. niger*, *A. oryzae*, *Fanscaeus*, *Rhizomucor*, *Kluyveromyces lactis*	Wheat bran and soybean cake
α-Amylase, β-amylase, glucoamylase	Strains of *Aspergillus* sp., *Rhizopus* sp., *Mucor* sp., *Bacillus* sp., *Saccharomyces* sp.	Wheat bran, rice bran, rice husk, coconut cake, tea waste, cassava, bagasse, banana waste, corn flour, saw dust, soybean meal, sweet potato, potato, rice hull, and sugar beet pulp
Glutaminase	*Vibrio costicola*	Wheat bran, rice husk, saw dust, and coconut cake
Inulinases	*Staphylococcus* sp., *K. lactis*	Wheat bran and soybean cake
Phytases	*A. ficuum*, *A. carbonarius.*	Canola meal
Tannases	*Rhizopus oryzae*	Wheat bran + tannic acid
Feruloyl para-coumaroyl esterase	*Penicillium pinophilum*	Wheat straw

been promising research using the vaccines for viral, bacterial, and parasitic diseases, several tumor types, etc., and eventually several DNA vaccines have been released for veterinary use. Among all only one DNA vaccine has been approved for human use, DNA vaccines may have a number of potential advantages over conventional vaccines, including the ability to induce a wider range of immune response type.

Table 2.7: Production of color, flavor, organic acid and other products using different microorganisms and substrates.

Products	Particular Constituents	Organisms Used	Substrates Used
Color	Orange pigment	*Monacus* sp.	Agroindustrial residue
	Pigments	*Monascus purfureus*	Sugarcane bagasse
	Carotenoids	*Penicillium* sp.	Corn meal
Flavor	2,5-DMP (Fruity aroma)	*B. natto*	Soybeans
	Fruity aroma	*Ceratocystis fimbriata*	Agroindustrial waste, cassava waste, apple pomace, soybean
	Acetaldehyde and 3-methyl butanol	*R. oryzae*	Tropical agrowaste residue
	Tetramethyl pyrazine (nutty and roasty flavor)	*B. subtilis*	Soybean
	Acetaldehyde and 3-methyl butanol	*R. oryzae*	Tropical agrowaste residue
	Strong pine apple aroma	*C. fimbriata*	Coffee husk
	Monoterpene alcohol and isoamyl acetate (fruity flavor)	*K. marxianus*	Cassava baggasse, giant palm bran
Organic acid	Lactic acid	*R. oryzae*	Sugarcane bagasse
	Lactic acid	*Lactobacillus paracasei*	Sweet sorghum
	Lactic acid	*Lactobacillus amylophilus GV6*	Wheat bran
	Citric acid	*A. niger*	Agroindustrial residue
	L-Glutamic acid	*Brevibacterium* sp.	Sugarcane bagasse
Gum	Xanthan gum	*X. campestris*	Apple pomace, grape pomace, citrus peels, spent malts, etc.
Biofuel	Ethanol	*Saccharomyces cerevisia*, *Schwanniomyces castellii*, *Zymomonus mobilis*, *Candida utilis*, *Tarula utilis*	Apple pomace, sorghum carob pods, sugar beet, sweet sorghum, sweet potato, wheat flour, rice starch
Vitamins	Vitamin B12, B6, riboflavin, thiamin, nicotinic acid, nicotinamide	*Citrobactewrfreundii*, *Klebsiella pneumoniae*, *Rhizopus oligosporus*, *R. arrhizus*, *R. stolonifer*	Soybean tempeh
Surfactant	Biosurfactants	*Bacillus subtillis*	Agroindustrial residues, molasses
Biocontrol agent	Biopesticides/ bioherbicide	*Entomopathogenic and Mycoparasitic fungi*	Sweet sorghum, rice flour, perlite-cornmeal agar
Antibiotics	Penicillin, cyclosporin, cephamycin, tetracyclin	*P. notatum*, *P. crysogenum*, *Tolypocladium inflatum*, *Fusarium solani*, *Neocosmospora varinfecta*, *Nocardia lactumdurans*, *Streptomices catteya*, *S. clauverigerus*, *Streptomyces viridefaciens*, *etc.*	Agroindustrial residue
Others	Gibberellic acid	*Gibberella fujikuroi*	Agroindustrial residue

2,5-DMP, 2,5-Dimethyl pyrazine.

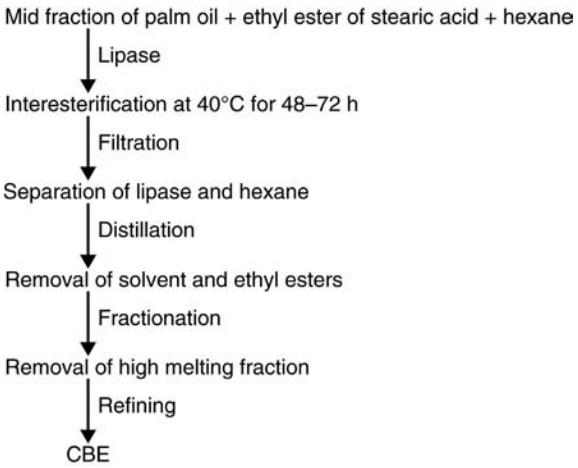

Figure 2.5: Manufacturing of Cocoa Butter Equivalent (CBE) Using Lipase.

3 Bioengineered Animals

Genetic engineering or bioengineering in animals can be defined as the deliberate changes in animal genome using the techniques of modern biotechnology. It provides various tools for improving animal welfare and health. Techniques, such as artificial insemination, embryo transfer, in vitro fertilization, cloning, etc. are used to improve the genetic makeup of animals. It provides various benefits, such as healthier offspring, healthier and safer food production from animals, consistent quality, disease resistance, etc. Nowadays, four techniques are being used to fabricate bioengineered animals, such as:

1. Transfer of nuclear material
2. Microinjection
3. Viral vector infection
4. Transfer of embryonic stem cell

3.1 Transgenic Dairy Cattle for Modified Milk

Bovine milk is well known as ideal food human as it contains balanced amount of vitamins and minerals, such as calcium and also good source of essential amino acids (Karatzas and Turner, 1997). An adult person can fulfill the calcium requirements by consuming two glasses of milk and milk products (Rinzler et al., 1999). Casein share majority, for example, 80% of total milk protein and is very nutritious (Brophy et al., 2003). αS1, αS2, β, κ casein determine the physicochemical properties of milk and any alteration in protein composition can affect milk's functional characteristics. Amount of protein content in milk significantly influence the cheese-making process, its yield, and nutritional

feature (McMahon and Brown, 1984). κ and β casein are the most important milk protein. Enhanced κ-casein content can decrease the size of the micelles, which provide improved heat stability while β-casein is more phosphorylated and connected to calcium phosphate, which affects calcium levels of milk (Dalgleish et al., 1989; Jimenez Flores and Richardson, 1998). The modification in milk composition is not limited to proteins only but can be extended to manipulation to the lactose, metabolic enzymes, milk fat and minerals in milk. Milk modification in transgenic animals have different applications; making it suitable for infants. Lacto-ferrin is an iron-binding protein has antimicrobial properties and may also mediate some effects of inflammation and have a role in regulating various components of the immune system. Its level in human milk is about 1 g/L (in human colostrum about 7 g/L). As the levels of lacto-ferrin in cow's milk is only about one-tenth that in human milk, this has caught the attention of those involved in designing human milk replacement formulas. Experiments are currently underway to add other naturally occurring human milk proteins—also having antimicrobial properties—and genes to alter the fatty-acid composition of milk and to make more healthier enriched mix suitable for heart patients.

3.2 Increased Muscle Growth in Cattle

Myostatin (MSTN) is a well-known growth differentiation factor 8 (GDF-8), a member of transforming growth factor β (TGF-β) family, releases myocytes, which inhibit myogenesis so decreased the muscle growth and differentiation. These are the families of animals that possess mutations with this gene, which display an enhanced muscling phenotype, a desirable agricultural trait. The myostatin gene is highly conserved in other species of animals, such as human, bovine, rat, murine, zebrafish, chicken, and turkey. The myostatin knockout mice have been developed with increased lean muscles mass, which enlarged the hip and shoulder of transgenic mice. The homozygous of animals of such knockout animals have achieved 2–3 times more muscle weight as compared to the normal ones. Myostatin may bind to NH_2-terminal or prodomain portion of the protein noncovalently with mature myostatin, eventually inhibition occur (Thies et al., 2001). Mature myostatin interfere due to over expression of prodomain (Yang et al. 2001).

3.3 Transgenic Swine With Reduced Fat Content

Bovine somatotropin, a growth hormone, is formed in the pituitary glands of young cattle and is well known for accelerating the milk production in lactating cows (Leury et al., 2003), though it is safe for human consumption. It gets inactivated during digestion in bovine gut making it biologically inactive for humans (Etherton, 1991).

Pursel et al. (1989) reported that recombinant bovine growth hormone (rBGH) caused decreased fat content in transgenic pigs. To introduce rBGH into pig genome microinjection

of pronuclear technique was adopted by controlling with mouse metallothionein-I (MT) promoter. First transgenic pigs were produced by artificial insemination with the sperms of rBGH transgenic males in nontransgenic females. The decrease in fatty acid content was noticed in transgenic pigs in comparison to the controls but there was no significant difference among the cholesterol levels. Thus, the consumers can get benefits from pork products with lower fatty acids, if the BGH secretion levels are controlled accurately (Solomon et al., 1994).

3.4 Transgenic Poultry: Egg as Bioreactors

Exogenous proteins are introduced to satisfy the consumer demand of various proteins used in biopharmaceuticals and it is produced with the help of egg, which was used as bioreactor in egg white (Gilbert, 1984). As the egg white is sterile in nature and has long shelf life and it is controlled by a single ovalbumin gene (Harvey et al., 2002; Tranter and Board, 1982). Harvey et al. (2002) introduced a bacterial gene β-lactamase from *E. coli* in egg white of transgenic chicken, expressed, and secreted. Expression of the gene was done using replication-defective retroviral vector introduced from avian leucosis' virus. Omnipresent cytomegalovirus promoter was used to exaggerate the expression. The protein β-lactamase was biologically active and the expression levels linger constant till fourth generation of transgenic hens. Though the levels of expression are far below those required for commercialization and an intense effort is underway to develop promoters driving much higher levels of expression but the results demonstrated the possibility of to use chicken's egg as a bioreactor.

3.5 Bioengineered Fish

Fish, an important protein source for the majority of people on the planet, is still primarily gathered from the wild, with serious consequences. Heavy investments into fishing fleets and technology, and ever-increasing yields, put the ocean's fisheries under increasing stress. Many fishing grounds are already overfished to the point that their future viability is threatened. GM fish have considerable potential to further increase the yield of fish farms but have prompted serious concerns both in Europe and the USA about the possible environmental impact on wild species. To overcome these concerns and address public resistance to biotechnology, it is therefore important to develop a sound, reliable and widely accepted method of estimating the potential for harm caused by GM fish escaping into the wild. For example, few companies have come forward for the commercialization of Atlantic salmon carrying growth hormone gene from Chinook salmon (Zbikowska, 2003). The main difficulty is the risk involved with the introduction of transgenic fish in the wild (Muir and Howard, 1999). According to Rasak et al. (1999) though there is no method till now for 100% sterilization, aforementioned obstacles could be avoided by sterilization of transgenic fish.

3.6 Improving Fish Growth Rate

To improve fish growth rates fish can be cloned and recognized from various fishes, by introducing fish growth hormones (Devlin et al., 1994; Du et al., 1992). Rahman et al. (1998) reported that at University of Southampton in the United Kingdom, a transgenic fish (*Orechromis niloticus*) has been generated and cloned. It is transformed with genes from several salmons. After research, the best results are found from the Chinook salmon growth hormone, gene that was microinjected to the fertilized fish egg. A successful integration and the transfer of transgenes to second generations have been reported. The growth rate of transgenic tilapia was found to be 33% higher than the wild type of tilapia, reducing farmer's production cost.

3.7 Increasing Antifreeze Property in Fish

Antifreeze proteins (AFPs) and antifreeze glycoprotein's (AFGs) can be introduced in the plasma of many fish species to incorporate antifreeze property that protects from freezing at subzero temperature in the cold water (Davies and Hew, 1990; Davies and Sykes, 1997). These proteins inhibit the formation of ice crystals of fish serum by lowering its freezing temperature (DeVries, 1984). These are important from aquaculture point of view because most of the fish, such as salmon and tilapia, cannot produce them naturally and thus cannot survive under low temperature environment in the northern Atlantic coast, which is very common in creating it a main obstacle for offshore aquaculture (Hew et al., 1995). The freeze tolerant salmon has been developed to cut down the cost of manufacture. There are two types of AFPs one-skin type small polypeptides and other liver type small polypeptides (Gong et al., 1996; Hew et al., 1986). For freeze tolerance capacity of Atlantic salmon, liver type AFP was used, the genes were injected and inserted in chromosomes of fertilized eggs.

The identical level of liver-specific expression and seasonal variation similar to those in winter flounder and protein activity were reported up to three succeeding generations of transgenic salmon. The expression levels and protein activity were observed up to three generations of transgenic salmon. Hew et al. (1999) reported that AFP levels in the blood of transgenic fish till three generation were lower (250 μg/mL) winter flounder (10–20 mg/mL) and therefore, inadequate to provide freeze resistance to the salmon. Research has been focusing on to discuss better antifreeze properties by altering gene structure.

4 Bioengineered Microorganisms

More than 5000 years, human beings have, consciously and innocently, made use of natural fermentation of a range of food items, which comprise bread, dairy products, alcoholic beverages, vegetable products, and meat products. But it was more in recent times, just in the last century, that researchers comprehend that the method of fermentation was done by the exploitation of microorganisms and that each microorganism accountable for a particular

food processing could be isolated and identified. Now, with superior bioengineering practice, it is possible to characterize the important food strains with high precision, isolation, and improvement of genes involved in the process of fermentation, and transfer desirable traits between strains or even between different organisms.

4.1 Elimination of Carcinogenic Compounds

One of the most important, commonly used, and very well known microorganisms is *S. cerevisiae*, known as Brewer's yeast in the food industry. In bread manufacturing, yeast is generally used as a leavening agent and during alcoholic beverage manufacturing, yeast is used as starter culture and grain residues or molasses are used as raw material for fermentation. During fermentation, occasionally undesirable products are produced. Recombinant DNA technology is the only solution. With the help of recombinant DNA technology, undesirable by-products can be removed by incorporating properties in the GM yeast strain. Ethylcarbamate or urethane is the unwanted by-product of foods and beverages during yeast fermentation. Ethylcarbamate or urethane is the probable carcinogenic material (Aldhous, 1990; Ough, 1976). Reduction of ethylcarbamate from alcoholic beverage is the main plunge of the alcoholic beverage industry; a large amount of investment has been done for the research (Dequin, 2001). The spontaneous reaction between ethanol and urea produces ethylcarbamate, which is the degradation product of grape arginine. In the presence of yeasts, which produces arginase and that catalyzes arginine degradation. If arginase is blocked, the reaction of arginine to urea can be prevented; therefore, ethanol to ethylcarbamate conversion can be stopped. A transgenic yeast strain was developed by inactivating *CAR1 gene*, which encodes for the enzyme arginase (EC 3.5.3.1) (Dequin, 2001; Kitamoto et al., 1991) to reduce the formation of urea in sake.

By incorporating an unproductive *CAR1* gene in the area of the arginase gene, the scientist developed the mutant yeast strain, flanked by DNA sequence homologous to the region of the arginase gene. The useless gene was incorporated into the active *CAR1 gene* through homologous recombination, in the yeast chromosome, and disturbed its function. As a result, urea was detached and ethylcarbamate was not produce during sake fermentation. The analogous tactic can be applied to remove ethylcarbamate from other alcoholic beverages including wine (Kitamoto et al., 1991).

4.2 Inhibition of Pathogenic Bacteria

During manufacturing of fermented food to enhance the security, hygiene, and proficiency in the food industry, the major thrust is to maintain the originality of the inoculating bacterial culture and also particular protective cultures (Gardner et al., 2001). Generally, inoculating or starter culture is used to instigate an industrial fermentation. Inoculating or starter culture is a mixture of particular microbial strain. The inoculating cultures offer the food a unique odor and consistency and protective culture helps to maintain the originality of the food by keeping unaltered properties, and also stop growing objectionable pathogenic organisms (Geisen and

Holzapfel, 1996). Though every time it is not possible but for convenience during fermented food manufacturing, the same microorganism should be used as starter and protective cultures. With the help of genetic engineering, better strains can be developed, which can be used as inoculating and protective cultures, therefore novel characteristics can be amplified and undesirable properties can be suppressed (Hansen, 2002).

The genetic engineering research increases microbial strains efficiency, improves process stability, and improves product safety during optimization of inoculating cultures (Geisen and Holzapfel, 1996). During the development of selected fermented foods, for example, mold-ripened cheese, degradation of lactic acid by fungal organism results increase of pH level (alkaline). *Listeria monocytogenes*, a food born pathogen, get suitable environment to proliferate in the alkaline *pH*. This type of circumstances can be prevented by using single starter culture, which has enhanced properties to inhibit the growth of such hazardous pathogens. The lysozyme (EC 3.2.1.17) can be used efficiently to prevent *Listeria* in food. Van de Guchte et al. (1992) incorporated the gene responsible for lysozyme formation in *Lactococcus lactis*, the bacterium strain. This bacterial strain expressed and secreted lysozyme at high levels after the genetic transformation. Lysozyme-encoding genes from *E. coli* bacteriophages T4 and lambda was cloned, in wide-host-range vectors and expressed in *L. lactis*. Biologically active lysozyme were produced and secreted by the transgenic *L. lactis* strains, indicates that transgenic *L. lactis* strains can be applied both as a starter and protective culture (Van de Guchte et al. 1992).

4.3 Natural Sweetener Produced by Microorganisms

Microorganisms can be extensively used in manufacturing and improvement of food product along with flavor enhancer in fermented food manufacturing practices. Nowadays, many of the methods use synthetic chemical additives for flavoring of food products (Vanderhaegen et al., 2003). Nowadays, consumers are very health conscious and aware of health hazards caused by harmful synthetic flavoring of chemicals; so they prefer foods with natural food grade flavors (Armstrong and Yamazaki, 1986; Cheetham, 1993). Among thousands of natural volatile and synthetic fragrances, only a few hundred are regularly applied to food manufacturing in industrial scale (Somogyi, 1996). Bioflavors can be extracted from (1) plant sources (2) specific bioengineered microorganisms by biosynthesis. Bioflavors production by fermentation provides certain beneficial effect, such as the process is cheaper when produced and applied in large-scale without depending on natural resources and plant material (Krings and Berger, 1998). The wood sugar xylitol, a white crystalline material, taste is similar to sugar, is made from abundantly available xylose, the plant sugar (Nigam and Singh, 1995). Insulin is not required for metabolism; therefore, it is suitable for diabetic patients (Pepper and Olinger, 1988). Xylitol can prevent tooth decomposition in children and adults as it provides prevention from *Streptococcus mutans*, the bacteria responsible for cavity formation in human. Sweetening index of xylitol and sucrose are 60 and 100, respectively and xylitol provides 40% less calories than sucrose. Metabolism of xylitol is very slow

and human body can utilize partially. Xylitol is FDA approved sweeteners. Xylitol is used as sweeteners in foods since 1960s in United States for special dietary purposes (Emodi, 1978). Due to its extensive use in fermentation industry yeast (*S. cerevisiae*) can convert xylose to xylitol. Therefore, it is used for the commercial production of xylitol. Govinden et al. (2001) introduced *XYL1* gene into *S. cerevisiae* isolated from *Candida shehatae*, novel xylose reductase (EC 1.1.1.21). The *XYL1* gene from *Candida* was cloned behind the *PGK1* promoter and the construct was introduced and transformed into yeast by electroporation into the yeast expression vector pJC1. Effect of cosubstrate was evaluated by using glucose, galactose, and maltose on xylitol production from xylose. When glucose was used as cosubstrate from 50 g/L of xylose, highest xylitol yield of 15 g/L was obtained.

4.4 Production of Carotenoid in Microorganisms

Carotenoid is a group of structurally different coloring body present in plants, animals, and microorganisms. These pigments have multiple numbers of biological functions; for example, it provides color, produce hormone, harvest light, and have photo protection properties (Campbell and Reece, 2002). Carotenoids are principally used as natural food colorants, vitamin supplements in animal feed, and nutraceuticals in the pharmaceutical industry. Current studies have suggested numerous nutritional health benefits from the consumption of carotenoids. Carotenoids (astaxanthin, β-carotene, and lycopene) are very popular due to high antioxidant properties, which prevent harmful diseases, such as cancers, cardiovascular, inflammation from arthritis, boost immune system, and provide relief from pain (Giovannucci et al., 1995; Jyocouchi et al., 1991; Miki, 1991). Pharmaceutical industry uses extract of carotenoids from plant sources. As the cell wall of Microalgae, such as *Haematococcus pluvialis* is thicker, extraction of carotenoid astaxanthin is not easy though it generates high amounts of β-carotene. Using GE tools, effort has been taken to generate carotenoid-rich organisms. *Candida utilis*, the edible yeast is "generally recognized as safe," is a potential organism for industrial carotenoid and large-scale production of glutathione peptides and has already being successfully accomplished in *C. utilis* (Boze et al., 1992).

Carotenoids are manufactured from farnesyl pyrophosphate, the precursor of carotene in microorganisms and plants. Carotenoids, lycopene, β-carotene, and astaxanthin were synthesized by recombinant gene and cloned. Miura et al. (1998) developed a de novo biosynthesis process in *C. utilis* where bacterial genes encode for enzymes in the biosynthetic pathway. Four *Erwinia uredovora* genes (*crtE*, *crtB*, *crtI*, *crtY*) and two (*crtZ*, *crtW*) genes from *Agrobacterium aurantiacum* were used for manufacture of four different plasmids. According to report, following designs were recommended:

For lycopene production, plasmid pCLR1EBI-3 containing *crtE*, *crtI*, and *crtB*; β-carotene plasmid pCRAL10EBIY-3 containing the genes *crtY*, *crtI*, *crtE*, and *crtB*; astaxanthin synthesis in a dual plasmid system for pCLEIZ1 containing *crtE*, *crtI*, and *crtZ for* pCLBWY1 containing *crtW*, *crtY*, and *crtB*.

By electroporation technique, these genes were incorporated into the yeast chromosome. Then the plasmids were linearized by restriction digest and transformed into *C. utilis*. It was reported that the resultant transgenic yeast produced considerable amounts of lycopene (1.1 mg/g dry weight), β-carotene (0.4 mg/g dry weight), and astaxanthin (0.4 mg/g dry weight).

Those were the equivalent quantity found in microorganisms, which produces these carotenoids naturally (Miura et al., 1998). It indicates that *C. utilis* has a great potential for use in large-scale production of commercially important carotenoids.

5 Detection Methods

To maintain the food safety, various methods have been developed to ensure the traceability of genetic material. The detection methods are used to achieve the consumer faith by ensuring their safety.

5.1 Transgene Detection Method

Transgenic food products can contaminate the nontransgenic material; therefore, protection is needed. One very popular example is the contamination of corn from bioengineered corns. Though such events can have negative impact on the consumer acceptance for GM foods, so to increase their potential achievement and approval, most influential, handy, and cost proficient method is real-time quantitative PCR (Higuchi et al., 1992). Principal rudiments used today for detection of GMOs is antibiotic resistance markers and promoters, but they are not always perfect since the same signature sequences can be found in multiple number of GMOs, and are also injurious for humans and atmosphere. European Union in 2004 has already been banned on the usage of such markers. Compulsory tagging of GMO foods through a 1% threshold level for the existence of transgenic material was established. R&D scientists from the German company, Icon Genetics, proposed a novel idea for worldwide recognition of GMOs (Marillonet et al., 2003). They proposed the standardized coding procedure for accumulating the nontranscribed DNA based technical information to transgene before its insertion to the genome. Nucleotide based triplet is the basis of coding, which consists of any of the 26 letters of latin alphabet, an Arabic numeral from 0 to 9, and then a space character, giving a total of 37 characters (Marillonet et al., 2003). The researchers could introduce biologically unbiased, nongenetic coding series that interpret into unique information, for example, the name of the company, production date, and place of production, product model, and serial number. PCR performance and sequencing of the fragment is needed to read the DNA-encoded information.

Another method to detect GMO involves distinctive genomic progression flanking the transgene. Thermal asymmetric interlaced-PCR approach is rapid and is a proficient method to magnify the indefinite sequences adjacent to the known incorporation sites. Supplementation specific primers are used along with the arbitrary degenerate primers, which are intended to fluctuate at their annealing temperatures.

Amplification of marker gene followed by real-time PCR performance with quantitative tool is the two consecutive steps for accurate DNA quantification. The quantitative tools are:

1. DNA binding dyes (Morrison et al., 1998),
2. fluorescent oligonucleotides (Whitcombe et al., 1999),
3. molecular beacons (Tyagi and Kramer, 1996),
4. fluorescence resonance energy transfer probes (Wittwer et al., 1997),
5. TaqMan probes (Heid et al., 1996).

The application of three oligonucleotides in PCR reaction turns out to be highly unambiguous. The detection process comprises amplification by primers and then detection by annealing. TaqMan probe provides quantifiable fluorescence light. The intensity of light confirms the quantity of target gene present in the food sample.

5.2 Food Pathogen Detection

Food poisoning occurs due to the presence of toxin producing bacteria, such as *Salmonella*, *Vibrio*, *Listeria*, and *E. coli*. O157:H7 is the most harmful strain of *E. coli*, which produces Shiga toxins (*stx1* and *stx2)*. These Shiga toxins injure the lining of large intestine causing severe diarrhea and dehydration eventually, which have an effect on other organs also and finally if absorbed in blood stream can be fatal (Riley et al., 1985). Mead et al. (1999) reported about 73,480 illnesses and 61 deaths in USA rooted from ground beef, unpasteurized milk, and roasted beef. These products if not preserved properly can contaminate and can be responsible for the transmission of food borne illness. PCR was used to detect the pathogen, as PCR is fast, sensitive, and reliable. Traditionally, PCR follows gel electrophoresis process and analyses very small number of samples during one run. PCR-ELISA approach, which is 100-fold rapid and more accurate screening for the detection of *E. coli* O157:H7 and other STEC were reported. With the help of robots, the process can be automated in near future and also permitted rapid, susceptible, accurate, and large-scale screening of microorganisms that produce the Shiga toxins. Using explicit biotin-labeled PCR primers, this method can also be applied to detect any other food pathogens.

6 Conclusions

Fabrication of herbicide, insects, and pathogen-resistant transgenic plants of many food crops have led to the enhanced production at low production cost. Three times faster growing transgenic salmon can be manufactured by introducing foreign genes in salmon. Development of engineered foods with better characteristics in terms of nutrition, taste, quality, and safety are the principle of the next generation modern biotechnology. Fabrication of new vegetable solid fat with lower amount of saturated fatty acids, production of more nutritious healthier meat, bioextraction of plant bioactive nutraceuticals, which provides excellent health benefits

but it is present in very low quantity in plant. Biotechnologically modified foods not only help to produce novel compounds, but also make improvements in existing ones, as well as provide food safety and security.

As a whole, biotechnology offers numerous innovative tools like new sensors to identify microorganisms and their toxin using number of biosensors, as well as DNA probes, PMCA, ELISA tests. PCR has been developed to detect the existence of infectious pathogenic agents, such as bacteria, virus, fungi, etc. It is proved that using biotechnology in modern food, variety of more nutritious, healthier, tastier, secure, durable, safe, and convenient food products can be delivered to the world at more affordable prices.

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