

# Chapter 12

## Application of Biotechnology in the Food Industry



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### Introduction

Biotechnology is a vast area of science and has exhibited pertinence in human development for centuries (Sugumaran & Ponnusami, 2017). Biotechnology is extended to various fields, including tissue culture, fermentation, DNA fingerprinting, selective breeding, and recombinant DNA technology (Daddiego et al., 2017; Kamle et al., 2017; Krasznai et al., 2017; Ledoux & Antunes, 2017; Lucarini et al., 2016; Nitschke & Silva, 2016). This technology is also involved in treating various infectious diseases and genetic disorders through the complete analysis of genes/DNA (Calvo-González, 2016; Hamad et al., 2017; Keskin et al., 2004; Lao et al., 2017; Šuster et al., 2017). Food processing, by definition, means to apply various operational methods and technologies in order to convert raw, bulky, and perishable, food materials into sustainable, and palatable food commodities (Swetwiiwathana & Visessanguan, 2015). Presently, there is a growing concern about low-cost production and wholesome food products of high value for improving the health of human beings.

The application of biotechnology in the food industry has a consequential effect on the living population. Biotechnology can potentially resolve the need for food and help to avoid mass starvation in the future. It is the field of increasing food productivity, enhancing its nutritional content and organoleptic properties.

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Biotechnology leads to the better utility of food by removing allergens and toxic components. It could also contribute to food security while assisting in promoting sustainable agriculture in developing countries. Globally, consumers are increasingly concerned about food safety and quality. The awareness of replacing chemical additives with natural ones has resulted in an increasing demand for food products enriched with bioactive compounds that would beneficially affect human health (Miguel et al., 2013). Therefore, in the present era, different gluten-free and dietary fiber-enriched food products and food products containing probiotics and oligosaccharides are easily found in the market (Patel & Goyal, 2011).

Lipase acts at the aqueous and a non-aqueous interface. When the water activity is low it synthesizes ester from glycerol and long-chain fatty acids. A “true lipase” splits long-chain fatty acids and emulsified esters of glycerine, e.g., tripalmitin and triolein. The applications of commercial enzymes have been increasing significantly in the last decades, particularly in the food industry (Anishetty & Gowtham, 2017). The biotechnological applications employing living organisms, or derivatives modify products for specific use. Yeast is utilized in fermented products, functional food, and nutraceutical production (Padilla et al., 2015; Rai & Jeyaram, 2017; Rai et al., 2017).

Functional foods by definition, are aliquot of a diet and possess health benefits in excess of their nutritional attributes, whereas nutraceuticals comprise purified components of food that holistically demonstrate health benefits. Genetically modified food (GMF) is also synthesized by using various measures of biotechnology. Modern Biotechnology is also termed transgenic or genetic engineering technology in which Nuclear DNA is altered by inserting a gene that encodes a desired trait and is termed recombinant DNA. The expression of recombinant DNA encodes desired product and when employed to improve food characteristics or yield is defined by the term food biotechnology (Morin, 2008). Modern Biotechnology enhances yield, shelf life, taste, and nutritional values, and also facilitates fermentation and enzymatic processes. In developing countries, biotechnology has the maximum potential to remove malnutrition, hunger, and various diseases in developing countries. Products of modern biotechnology are reasonable at a commercial level and therefore can improve food and agriculture, which would result in an increase in the economic status of farmers as well (Adenle, 2011).

## **Biotechnology in Food Industries**

In food processing industries, biotechnology is profound for the better productivity of food products. The various aspects of biotechnology employed in food processing sectors encompass increasing the food yield, improving the nutritional value of food products, using the fermentation process to produce different products, improving their shelf life, and organoleptic properties, and enhancing food safety (Lokko et al., 2018; Nguyen et al., 2017).

### ***Biotechnology for Better Yield***

Transgenesis is done with the aid of biotechnology in which a gene of one organism is manipulated into another of a similar or distinct species to result in the gene expression which is then transferred to the next generation (Song et al., 2017; Zhu et al., 2016). Rats, mice, sheep, cows, rabbits, etc., are some of the examples (Srinivasa & Goswami, 2007). Genetically engineered salmon, a fish, is disease resistant and possesses improved tolerance to environmental stresses (Dunham & Su 2020; Forabosco et al., 2013). The United States Food and Drug Administration approved it as a safe and healthy food for human consumption. Incorporation of extra gene copies that encode  $\kappa$ -casein and bovine  $\beta$ - to female bovine fibroblasts revealed an 8–20% increase in  $\beta$ -casein in milk produced from such animals and an altered  $\kappa$ -casein to total casein ratio (Brophy et al., 2003). In poultry, growth traits have been observed to be associated with polymorphisms in the growth hormone, ghrelin, lambr1, growth hormone receptor, MC3R, IGF-II, MC4R, and TGF- $\beta$  (Fang et al., 2007; Huang et al., 2007; Jiang et al., 2002; Li et al., 2002; Qiu et al., 2006; Yan et al., 2002). Genetically modified food technology has been adopted for commercial production of GMF and allergenicity tests, digestion, and genetically modified food (GMF) toxicity. Biotechnology has aided scientists in producing GMF with better taste and the seeds eliminated from such food articles resulted in more soluble sugar and enhanced sweet taste (Falk et al., 2002). Biotechnology has modified the pathways of fermentation pathways to add aroma.

### ***Improvement in the Nutritional Value of Foods***

With advancing biotechnology, food bio-fortification by recombinant DNA technology and fermentation processes is becoming advantageous in food industries (Cashman & Hayes, 2017). The term Designer food, which was introduced in Japan in 1980, is defined as processed food that contains nutrients resulting in additional benefits of health besides its nutritional status. Cruickshank started the approach of designer eggs, and by making the feed interventions, he observed the modification of fatty acid composition in the yolk of the egg. The designer eggs adorned with omega-3 fatty acids exhibited better polyunsaturated fatty acid stability during the cooking of egg and storage, and high availability of nutrients such as carotenoids, vitamin E, and selenium improved omega-3 and antioxidant content in people consuming eggs (Surai & Sparks, 2001). Several research scientists have developed a wide range of designer eggs, which have been observed to contain omega-3 fatty acids and antioxidants (Sim & Sunwoo, 2002). Raes et al. developed a designer egg supplemented with linoleic acid (Raes et al., 2002). Eggs supplemented with vitamin A and  $\beta$ -carotene have also been developed. By removing the  $\beta$ -LG gene from bovines, allergy to cow milk in children can be minimized (Sabikhi, 2007). Researchers have also recommended that chicken and beef enriched with selenium

can be produced by incorporating organic selenium poultry and farm animal feed (Fisinin et al., 2009). Functional foods are fathoming much importance by playing a significant role in preventing diseases and promoting health benefits (Fisinin et al., 2009).

### ***Biotechnology in Fermentation***

In fermentation processes at a commercial level, starter cultures have been utilized to produce different food products of high value (Holzapfel, 2002). Restricting activity of these cultures has been observed due to various substances such as hydrogen peroxide, bacteriocins, diacetyl, and organic acids (Hutkins, 2006). Plasmid transfer, cloning, protoplast fusion, and transduction of the starter cultures have been employed to enhance the anti-cholesterolemic properties, resistance against enteropathogens, and anti-carcinogenic activity of livestock foods. Fermented milk products positively affect the intestines and possess good health benefits (Berni Canani et al., 2017). In fermented dairy and meat product preparation, strains of lactobacillus can be potentially used as probiotics (Pennacchia et al., 2006). Fermented foods are value-added products that are rich in nutrients, prolonged shelf life, are easy to digest, and are more beneficial for the intestinal tract. Thus, biotechnological measurement can be employed to yield enhanced bacteria, yeast, and mold strains, utilized for preparing fermented dairy and meat products.

### ***Biotechnology for Increasing the Shelf Life of Food***

The food shelf life is being enhanced by bacterial fermentation. Most fermentation processes involve the sugar conversion by lactic acid bacteria to lactic acid. In the present era, lactobacilli are gaining attention due to bacteriocins production (Collins et al., 2017). These constituents can be used as a natural preservative in the food industry. Lactic acid bacteria and their metabolized products are generally considered safe (GRAS) (Patel & Prajapati, 2013). Bacteriocins can be obtained by harboring specific bacterial cultures in a controlled environment. Nisin has been officially utilized in the food industries and approved worldwide for its utilization (Kaškonienė et al., 2017). The direct addition of nisin to food products, including cheese, flavored milk, canned foods, etc., has also been permitted. A multitude of refrigerated vacuum-packaged processed dairy, fish, meat, and vegetables contain strains of Lactobacillus, Leuconostoc, Brochothrix, Clostridium, and Carnobacterium (Rodríguez et al., 2002). They get multiplied at refrigerated temperature and cause product spoilage. Reduction in Listeria count has been achieved by adding a culture of Lactobacillus sakei in chilled cured pasteurized sliced vacuum-packaged meats and chilled raw ground meat (Devi & Halami, 2011).

### ***Enhancement in Organoleptic Properties of Food***

The organoleptic property of food plays a significant role in the fathomable acceptance of food products. Biotechnology has a major role in the evolution of chemical, nutritional, technological, and organoleptic properties (Smaldone et al., 2017). Microbial culture in food production can also enhance the organoleptic properties of food product. A study has estimated that more than 100 commercial chemicals of aroma have been derived by using biotechnological measures (Berger, 2009). The organoleptic attributes of fermented food products are profound, in terms of color, taste, flavor, and aroma (Singh et al., 2012). The Recombinant DNA technology has enhanced efficiency in non-nutritive sweetener production such as aspartame and thaumatin (FAO, 2010). The lactic acid bacteria are used in producing a diverse range of food products. *Lactobacillus delbruekii*, *Lactobacillus helveticus*, and *Streptococcus thermophilus* produce diacetyl compounds that produce flavor and are employed to produce acidophilus milk, yogurt, and high-scalded cheese.

### ***Biotechnology in Food Safety***

The European Food Safety Authority has proclaimed that bacteria used in the production of feed carry acquired resistance genes that might jeopardize the living population (EFSA, 2007). Ensuring food safety is important to provide appropriate safeguards for a consumer and encourage trade. Contamination of microorganisms is monitored in the final product and during the production process, sanitation, and cleaning and is one of the important factors in the process of manufacturing in food and biotechnology (Ochoa & Harrington, 2005). Genomics and proteomics technology provide more specific methods for checking microbial contamination of food. Various tools of biotechnology, including PCR (polymerase chain reaction), genetic engineering, amplified fragment length polymorphism, recombinant DNA technology, random amplified polymorphic DNA (RAPD), etc., are being used, and they tend to aid in the authentication of meat and checking its speciation. Development and expansion of new methods for evaluating high-risk pathogens in food products are enormously crucial in the context of food safety (Naveena et al., 2017).

### **Oligosaccharides**

These are the carbohydrates that are the polymers of monosaccharides linked by glycosidic linkages. They have a wide application in the food industry. Oligosaccharides are obtained naturally and chemically or through various biotechnological processes (Pinelo et al., 2009; Villares, 2010). Amongst the various functions, their prebiotic potential is one of the attention-seeking attributes. An

oligosaccharide is prebiotic, not to be absorbed or hydrolyzed in the upper part of the gastrointestinal tract, and therefore undergoes assimilation selectively using a multitude of microorganisms in the colon that promote systemic or luminal benefits. Microorganisms must be safe, multiply and colonize the tract, and be able to survive through the tract (Rioux et al., 2005; Roberfroid & Prebiotics, 2008).

Oligosaccharides like fructo oligosaccharides (FOS) and galacto oligosaccharides (GOS) have been extensively analyzed for their prebiotic benefits. FOS is present in little quantity in natural sources, including sugar beet, asparagus, onion, garlic, wheat, Jerusalem artichoke, banana, honey, tomato, etc. (Sangeetha et al., 2005b). Their large-scale production is also restricted by seasonal conditions from natural resources (Sangeetha et al., 2005a). With the consumption of FOS, various health benefits are associated, including colonic microflora modulation, activation of the immune system, gastrointestinal physiology improvement, facilitation of mineral availability, reduction in serum cholesterol, triglyceride, and phospholipid level, and prevention of colonic carcinogenesis (Charalampopoulos & Rastall, 2012; Lopez et al., 2000; Nemukula et al., 2009). FOS can be employed as a non-carcinogenic and zero-calorie sweetener. 1-Kestose is observed to increase the power of sweetness in comparison to other sc-FOS, and 1-ketose-rich sc-FOS syrups can potentially replace sugar for diabetic patients (Mabel et al., 2008).

Several GOS are considered prebiotics because of their undigestible nature and can be selectively used in the human intestine, thus improving human health (Oliveira et al., 2011; Villamiel et al., 2014). GOS applications are slowly increasing globally because of their significant health benefits. They are present in bakery products, yogurt, beverages, etc. (Venica et al., 2015). GOS potentially stimulates the bifidobacteria and lactobacilli growth in the lumen. GOS can prevent bacterial adherence as they exhibit the property of camouflaging the receptors of host cells in which the adhesion of bacteria occurs (Nauta et al., 2010). They can prevent colon cancer development since they can delay fermentation and reduce genotoxic bacterial enzyme activity associated with this disease (Shoaf et al., 2006). They can stimulate mineral absorption, and their calcium absorption effects have been affirmed. They can alleviate constipation, a common chaos in elders and expecting women. Furthermore, GOS indirectly acts on systemic and mucosal immune activation and protects against allergic manifestations.

Isomalto-oligosaccharides (IMO), including malto-oligosaccharides, are produced from starch. IMOs naturally occur in numerous fermented food products and sugars such as honey, soybean sauce, etc. IMOs exhibit mild taste and are relatively inexpensive. They exhibit relatively low viscosity, less sweetness, and bulking characteristics. They were also utilized as a sugar substitute for diabetics and to prevent dental caries (Zhang et al., 2010) Among the prebiotic oligosaccharides, IMOs are largely employed in food industries. They are widely used as food ingredients (Bharti et al., 2015). IMOs are commonly used for being highly stable, available, and cost-effective (Nguyen & Haltrich, 2013). The benefits of IMO consumption have been evaluated in some studies that investigate health conditions in specific population. IMOs have been observed to stimulate bowel movements and reduce total cholesterol level with an intake of 10 g/day in elders (Meyer, 2015).

## Enzymes in Food Processing

Enzymes are being utilized in food production and processing at the industrial level. Food processing industries have used enzymes produced through genetically modified organisms for decades. These enzymes comprise proteases and carbohydrases. To get a higher production of such enzymes in a short period, cloning has been done to the genes. These types of enzymes are used in cheese, and curd making, as well as for food flavoring items. A high amount of such enzymes is utilized in food industries. In the United States, more than 50% of proteases and carbohydrases are used in the food industry. They comprise renin and  $\alpha$ -amylase. Some of the enzymes that are genetically modified and used in food industries are mentioned below:

- Catalase in the production of mayonnaise and in removing hydrogen peroxide
- Chymosin as a milk coagulant in the cheese production
- Glucose oxidase in baking to stabilize the dough
- $\alpha$ -amylase in converting starch into maltose and in sweetness baking
- Protease in meat tenderness and in baking and milk products

Enzymes have been used for cheese production and indirectly through yeasts (Schmid et al., 2001). Pectinases, containing various enzyme activities, have been used in fruit juice manufacturing that aid in clarifying juice. Microbial enzymes are majorly used in starch industries. The starch hydrolysis has been substituted by glucoamylases and  $\alpha$ -amylases, converting approximately 95% of starch to produce glucose. Xylanase and cellulase enhance the juice extraction from the pulp in addition to pectinase. Pectinase and amylase are used in the juice clarification. The various applications of different enzymes in the food processing sectors is listed in Tables 12.1 and 12.2 (Shakuntala et al., 2009).

Microbial enzymes are widely employed in the food processing industry. The worldwide  $\beta$ -galactosidase production has been enumerated at approximately 5.749 million MT per year. It helps in removing lactose from milk and producing galactosylated products (Husain, 2010). Research studies have shown that nearly 70% of the worldwide population of different age groups is not able to digest lactose. However, a low intake of dairy products besides being rich in calcium leads to a higher risk of fractures, such as osteoporosis. Lactose hydrolysis in milk before consumption has been observed to aid monosaccharide absorption. New technologies have been developed to produce dairy products that are lactose-free, including lactose hydrolysis. Thus,  $\beta$ -galactosidase hydrolytic activity in the food industries has fathomed global acceptance in the decades for the lactose reduction in milk trans-glycosylation reactions for GOS synthesis in a few past years (Oliveira et al., 2011; Park & Oh, 2010).

Hydrolysis of lactose has acquired much significance from technological, clinical, and environmental perspectives. Disaccharide hydrolysis increases the solubility of lactose and the product sweetness due to galactose and glucose thereby using a greater quantity of constituents of whey.  $\beta$ -Galactosidases can be produced from yeasts, bacteria, and filamentous fungi (Oliveira et al., 2011).  $\beta$ -Galactosidase

**Table 12.1** Enzymes used in food processing industries

Industry	Enzyme
Butter oil and butter	Lipase, glucose oxidase, catalase
Cheese	Lipase, proteinases, rennet
Animal feed	Glucanase, amylase, pentosanases, glucoamylases, cellulases, xylanases, proteinases, phytases
Biscuits	Amylases, hemicellulases, cellulases, pentosanases, proteinases
Bread	Amylases, cellulases, amyloglucosidases, glucanases, glucose oxidase, hemicellulases, proteinases, pentosanases, lipases
Brewing	Decarboxylase, acetolactase, amyloglucosidase, amylases, glucanase, cellulase, lipase, proteinase, pentosanase, and xylanase
Coffee	Galactomannanase, cellulase, pectinase, hemicellulases
Confectionery	Amylase, invertase, pectinase, proteinase
Egg processing	Proteinase, lipase phospholipase, catalase, glucose oxidase
Dairy products	Lactase, sulphhydryl oxidase, proteinase, lysozyme, lactoperoxidase, peroxidase, and catalase
Flavor	Glucanase, proteinase, peptidase, lipases, esterase, amylase
Fat	Esterase, lipases, glucose oxidase
Fish	Proteinase
Cloudy juices and fruits	Proteinase, amylases pectinases, cellulases
Fruit extract	Anthocyanase
Fruit and vegetable processing	Cellulases, macerating enzymes, pectinases
Tea	Cellulase, glucanase, pectinase, tannase
Protein	Glucanase, amylase, cellulase, pectinase, protease, hemicellulase
Starch	Amylase, cellulase, hemicellulase, isomerase, glucanase, lipase, pectinases, proteases, phospholipase

Shakuntala et al. (2009)

**Table 12.2** Enzymes in food as additives

Enzyme	Application
$\alpha$ -amylase	Ethanol fermentation, starch syrups, animal feed
$\beta$ -amylase	Maltose syrup, brewing
Cellulase	Animal feed
$\beta$ -glucanase	Brewing
$\beta$ -glucosidase	Transforming isoflavone phytoestrogens in soymilk
Dextranase	Dextran hydrolysis
$\alpha$ -galactosidase	Increased yield of sucrose; potentially used in the sugar beet industry
Invertase	Manufacturing inverted syrup from cane sugar
Lactase	Eliminating lactose from milk and milk products
Pectinase	Fruit processing
Protease	Baking goods, brewing, protein processing, meat tenderization, distilled spirits

Shakuntala et al. (2009)



exhibits various functional characteristics which could be further enhanced by isolating microorganism strains, yielding immobilized enzymes that are receptive to chemical mutagenesis, improved secretion of enzymes, and expression of genes by recombinant DNA techniques are continually increasing in the present era. Recombinant DNA technology is currently used in expressing and optimizing  $\beta$ -galactosidase production from the most diverse sources. Moreover,  $\beta$ -galactosidases enriched with properties such as higher yield of product, reduced inhibition of product, etc. could also be produced by using protein engineering techniques (Gosling et al., 2010).

## **Fungi in Processed Food**

Fungi comprise an impartial part of food, both as animal and human food. Single Cell Protein (SCP) is termed as a group of various microbial products of fermentation and can be employed in the fermentation of several waste effluents, such as wood, straws, food, and their processing wastes, alcohol, and residues of human and animal excreta. They are commonly produced as diluted solutions comprising less than 5% of solids, that undergo precipitation, filtration, centrifugation, and coagulation. Material stabilization for storage water removal is a mandatory step. SCP must contain about 10% moisture content or need to be acidified and condensed to keep spoilage at bay. It should be fed immediately after its production. Nevertheless, yeasts have better utility compared to other types of fungi (Shakuntala et al., 2009).

### ***Fungi: An Alternative to SCP***

Mycoprotein, namely Quorn is the most notable processed fungal food. The product is available as high-protein SCP flour. It has been developed as a substitute for meat on the basis of its organoleptic properties. In a larger air-lift fermenter the mycelium is continuously cultured. It has a filamentous structure that tends to induce the fibrous attribute of meat associated with the fungal nutritive value. The product is a less-calorie, low-fat healthy food and is also free from cholesterol (Shakuntala et al., 2009). The various uses of processed fungal food are mentioned as follows.

## **Application in Fermentation-Based Food Industries**

Macro fungi cultivation has flourished for a few years, but the fresh mycelia used as food have still not gained popularity globally. Furthermore, the function of such organisms, particularly yeast in the production and processing of food is indispensable. Fungal cell factories are greatly used in bread-making and brewing industries as they secrete a wider range of enzymes into the culture medium (Shakuntala et al., 2009).

## **Cheese and Bakery Products**

Bakeries particularly comprise wheat flour mixed with water, sugar, and salt, using yeast as a leavening agent at the incubation temperature of 25 °C. The yeast aids in the fermentation of sugar and produces alcohol and carbon dioxide. The liberated gas forms bubbles by the extension of gluten in the flour. During baking evaporation of alcohol occurs. Bread flavour and texture are estimated by various factors, such as amount of gluten in the flour, the length of leavening, the constituents of grain, and the temperature. The appearance of mycelium is a part of moldy cheese favorites amongst gourmets in the production of cheese. Camembert and Roquefort also termed blue cheese are synthesised by two species of *Penicillium*, *P. roqueforti* in Roquefort cheese and *P. camemberti* in Camembert cheese (Shakuntala et al., 2009).

## **Other Food Products**

A multitude of research has been done on various fermented products of food that lead to the establishment of the fungus identification included in the process, such as shoyu, miso, tempeh, and tofu. In spite of this, various microorganisms, such as bacteria used in the major fermented food products are unidentified. In Western culture, yeasts usually play a role in the fermentation, whereas the East has used a multitude of mycelial fungi. Among the various Asian food products soya sauce is one of the most familiar. Shoyu is a flavor enhancer. Tempeh is considered to have its origin in Indonesia and is prepared from the fermented legume seeds using *Rhizopus oligosporus*. Miso, a Japanese word given to fermented paste of soybean is consumed as a soup base or used as a flavour enhancer. During its fermentation, rice is washed, polished, steamed, and inoculated with *Aspergillus oryzae*, which results in the formation of rice koji which is further inoculated by bacteria and yeast and proceeds to fermentation (Shakuntala et al., 2009).

## **Fungi in a Regulated Diet**

The fungal food consumption has increased globally in recent years with increase in public demand for health concerns. Vegetarians have resorted to consuming freshly cooked mushrooms, beverages, and dietary supplements that are of fungal origin (Shakuntala et al., 2009).

### ***Fruiting Body Utilization***

The fruiting body of mushrooms has been consumed as fresh or in processed form and as a delicacy. Fungi can be technically produced through fermentation, media preparation, inoculation, and incubation. The culture media tends to be available in the form of substrates from sources of low value, including agricultural and industrial waste, and are transformed into food products of high value. Therefore, the utilization of fungi is economically important as well as eco-friendly. A multitude of edible mushroom species exist wildly approximately 19 species are widely used as food and nearly 8–10 are cultivated on a regular basis. The most common edible species, like *Agaricus bisporus*, when small are sold as button or portobello mushrooms when larger, and used in soups, and various dishes. Most of the fungi of Asian origin are being cultivated at a commercial scale and have been popularised in the West. They are also available in markets, such as grocery stores, which include oyster mushrooms, straw mushrooms, enokitake, and shiitakes. There are various other fungi like milk mushrooms, morels, truffles, porcini mushrooms, and black trumpets also known as king boletes, which are costly. The most common edible macrofungi are given in Table 12.3, exhibiting their medicinal and nutritional attributes, and can be utilized as a substitute for non vegetarian protein sources (Shakuntala et al., 2009).

### **Biotechnological Applications of Yeast in Food Industries**

Yeasts highly participate in producing various types of nutraceuticals and functional foods (Padilla et al., 2015; Rai et al., 2017). Functional foods, part of a normal diet are conventional commodities and exhibit various health benefits in excess of their nutritional attributes. Nutraceuticals comprise purified components of food that are proven to evince health benefits. Yeasts enhance bioactive components in fermented food by producing enzymes and metabolites, or they act synergistically with other classes of microorganisms to improve their functional characteristics (Rai et al., 2016). Yeasts are widely applied in functional food industries, such as

- Living cells can be utilized as probiotics
- Cell wall components, such as  $\beta$ -glucan exhibiting nutraceutical value
- Extracellular fractions that are secreted consist of folate, carotenoids,  $\gamma$ -amino butyric acid
- Specific enzyme producers are the key players in the biotransformation of food metabolites that result in producing nutraceuticals of high value (Padilla et al., 2015; Rai et al., 2016)

**Table 12.3** Common edible macrofungi with nutritional and medicinal properties

Fungi	Nutritional properties	Medicinal properties	References
Straw mushroom	Source of antioxidants because of the presence of $\beta$ -carotene in high quantity	Contains FIP-Vvo, that stimulates TH1 and TH2-specific cytokine	Cheung et al. (2003)
Winter mushroom	Contains mannofucogalactan which is a hetero galactan derivative of Flammulina that possesses nutritional attributes	Causes production of antibody by modulating the differentiation and function of TH-cell	Carbonero et al. (2008)
Oyster mushroom	Exhibits flavor and aroma; rich in carbohydrates, protein, fiber, vitamins, and minerals	Exhibiting antiviral hematological, antibiotic, antitumor, immunomodulation, and antibacterial attributes	Cohen et al. (2002)
Truffle	It has a tempting aroma and taste and is economically the desired delicacy	Therapeutic having anti-cholesterolaemic, anti-carcinogenic, viral, and prophylactic properties regarding to hypertension and coronary heart disease	Carbonero et al. (2008)
Reishi	It is employed in dietary preparation. Protein includes about 7.29% of dry weight. Metals and glucose account for about 10.21% and 11% of dry mass respectively, including Mg, K, Ca and Ge, being in major quantity	GLIS stimulates the activation of B lymphocyte, proliferation, differentiation, and immunoglobulin production	Bao et al. (2002)
The common morel	Morels are a feature of many cuisines, such as provencal	Antioxidant and scavenging activity, reducing power, and chelating effect; also comprises galactomannan inducing macrophage activity	Duncan et al. (2002)
The black morel	Rich in Vitamin D2	It is believed to cure the common cold, tuberculosis and high blood pressure	Mattila et al. (2000)
The half-free morel	The spongy texture of young morels is used to make delicious dishes	The ethanolic extract of Morchella has 85% antioxidant properties	Carbonero et al. (2008)

Shakuntala et al. (2009)

### ***Baker's Yeast***

The baker's yeast production is largely used for various purposes in food processing. It is a *Saccharomyces cerevisiae* strain that is selectively used to produce abundant gas of desired flavor. The organisms and bread dough are mixed to vigorously initiate the fermentation of sugar. The liberation of carbon dioxide gas during fermentation leads to the leavening of the dough (Shakuntala et al., 2009).

### ***Yeasts Used as Probiotics***

The various qualities needed for a microorganism to be considered probiotics include growth at high pH, capacity to withstand bile juice, hydrophobicity surface of the cell, and auto-aggregation (Fadda et al., 2017). Various research studies have been done on probiotics in the bacterial system, however, yeast has evinced its capability as a probiotic (Saber et al., 2017a, b). Yeasts are advantageous over bacteria since they are nearly 10 times bigger than bacteria and tend to resist antibiotics during antibiotic treatment. Yeast isolated from fermented food products has also been analyzed for its ability in the assimilation of cholesterol and its probiotic effect. A probiotic yeast, *K. marxianus* CIDCA 8154 has been observed to reduce oxidative stress and inhold an anti-inflammatory effect (Romanin et al., 2015).

### ***Constituent of Yeast Cell Wall as the Ingredient of Functional Food***

Yeast cell is a source of fiber and  $\beta$ -glucan that enhances immunity, reduces blood cholesterol level, and exhibit anti-inflammatory effect (Vieira et al., 2016). The yeast cell wall composition changes accordingly with various genera and is dependent on various conditions of growth, affecting the functional characteristics of its polysaccharide (Galinari et al., 2017; Jaehrig et al., 2008). The composition of cell wall of *S. cerevisiae* has been observed to comprise  $\beta$  (1  $\rightarrow$  3)-D-glucan (50–55%),  $\beta$  (1  $\rightarrow$  6)-D-glucan (5–10%), mannoprotein complex (35–40%) along with chitin (2%) (Kwiatkowski, 2009). Yeast beta-glucans, also called *Saccharomyces*  $\beta$ -glucans have been approved by EFSA for their role as novel food ingredients with available range between 50 and 200 mg (EFSA, 2011). In fermented foods, constituents of yeast cell wall are a potentially produce bioactive molecules that impart functional attributes to the product.

### ***Nutraceutical Production from Yeast***

Yeast is well known for nutraceutical production that prove to be an important part of the food industry, including folate, carotenoids, and  $\gamma$ -aminobutyric acid (GABA) (Chen et al., 2016; Greppi et al., 2017; Han & Lee, 2017). Products fermented by yeasts have been observed to comprise various biologically active metabolites that improve the product functionality. Yeast has been observed to produce GABA by glutamate decarboxylase (Han et al., 2016; Han & Lee, 2017). Folate comes under the category of essential cofactors in various biochemical reactions, and its poor availability in the diet has become one of the concerns worldwide (Greppi et al., 2017; Korhola et al., 2014). Yeasts that are used for producing functional foods have

resulted in fermented foods rich in folate (Hjortmo et al., 2008a, b; Kariluoto et al., 2006). Carotenoids are naturally pigmented compounds and play a crucial role in the food industries as they prevent oxidative stress-related diseases (Chen et al., 2016; Mannazzu et al., 2015). Carotenoids produced by yeast include  $\beta$ -carotene, astaxanthin,  $\gamma$ -carotene, torularhodin and torulene (Mannazzu et al., 2015; Moline et al., 2012). A non-proteinaceous thiol peptide, glutathione is able to diminish the negative effects of oxygen radicals, which makes it one of the phenomenal components for the application of nutraceuticals (Liang et al., 2009; Musatti et al., 2013). The widely studied yeasts, *S. cerevisiae* and *Candida utilis* have been observed to produce glutathione (Liang et al., 2009; Musatti et al., 2013). In fermented products, the inclusion of strains of yeast to produce these metabolites tends to have a positive influence on the consumption level (Rai et al., 2018).

### ***Yeast in Biotransformation to Produce High-value Nutraceuticals***

During fermentation processes, a wide range of biochemical changes occur due to the production of enzymes depending on substrate-specific nature (Rai et al., 2017). These changes result in the hydrolysis of the complex substrate to a simpler one and the transformation of the biomolecule into its active state. The output depends on the microorganism strains used in the process of fermentation and the composition of the substrate (Rai et al., 2017). Food components and yeast interaction result in the production of various types of metabolites that exhibit specific benefits on health depending on the biochemical composition of the product. Yeast-fermented food metabolites have been reported to possess free polyphenols, bioactive oligosaccharides, and biologically active peptides (Rai & Jeyaram, 2017). In the production of functional food, the yeast association with filamentous fungi positively affects various fermented products and bioprocesses important at the industrial level for nutraceutical production (Feng et al., 2007). Yeast has proven to be an integral part of fermentation associated with filamentous fungi for producing highly pure oligosaccharides exhibiting prebiotic attributes (Guerrero et al., 2014; Nobre et al., 2018; Sheu et al., 2013). Yeast associated with filamentous fungi is a promising opportunity for the development of functional foods (Rai et al., 2018).

### **Applications of Lipase in Food Industries**

Lipase is a group of hydrolases that takes part in triglyceride hydrolysis to yield glycerol and free fatty acids. Lipase is employed in two different ways. It is used as a biocatalyst in the making of food ingredients and is employed in the development of fine chemicals. Lipase is commonly used in food processing, oil and fat processing, textile, leather, degreasing formulations, detergents, pulp, paper processing,

**Table 12.4** Lipase enzymes in food processing industries

Industry	Role	Application
Dairy	Fat hydrolysis, butter fat modification, ripening of cheese	Flavoring agent development in milk, butter, and cheese
Bakery	Enhancement of aroma and flavour	Increment in shelf life
Food dressing	Quality enhancement	Mayonnaise and dressings
Dietary foods	Transesterification	Dietary products
Fish and meat	Flavour enhancement	Fat removal of fish and meat
Oil and fat	Hydrolysis and transesterification	Fatty acids, cocoa butter, margarine, mono, and diglycerides
Processing of tea	Lipid breakdown and flavour enhancement	Black tea

Joo et al. (2002)

production of pharmaceuticals, synthesis of fine chemicals, cosmetics, etc. (Houde et al., 2004). The utilization of lipase in food industries is to modify and hydrolyze biomaterials. Most commercially produced lipase enzymes are used for flavor enhancement in milk products and other food processing, such as vegetables, meat, fruit, baked foods, etc. A diverse role of Lipase enzymes in food processing industries is shown in Table 12.4 below.

### ***Lipase in the Dairy Industry***

Lipase is commonly used in the dairy industry for the hydrolysis of milk fat. In the dairy industries, lipase enzyme is used to alter fatty acid chain length and in the flavor enhancement of various types of cheese. The other applications of lipase in the dairy industry include increasing cheese ripening and fat, butter, and cream lipolysis (Sharma et al., 2001). The free fatty acids produced by lipase activity on milk fat produce soft cheese exhibiting specific flavor attributes. Pre-gastric tissues and pancreatic glands of young ruminants, including calf and lamb are traditional sources of lipases used in the enhancement of cheese flavor (Aravindan et al., 2007). Gastric lipase has been used in accelerating the development of flavor and cheese ripening, e.g., provolone, cheddar cheese, and ras cheese.

The addition of lipase has been reported to enhance the rate of liberation of fatty acid, which tends to accelerate flavor development. The research study has shown that the supplementation of calf lipase and the increase in the ripening temperature in the range 7–53 °C resulted in an increase in the liberation of fatty acids. However, the lipase continued to remain in its active form after ripening which could cause the development of a strong rancid flavor. The addition of a high concentration level of lipase enzyme during ripening of cheddar cheese tends to result in increased enzymatic reactions that could deteriorate the desirable attributes and thereby decrease the yield. The liposome technology adaptation to accelerate the ripening of cheese has been observed to reduce bitterness and yield losses.

### ***Lipase in the Bakery Industries***

In bakery industries, a high focus on lipase enzymes is of great concern. Various research findings have suggested that lipase enzyme can be used in substitution to traditional emulsifiers it potentially degrades wheat lipids in order to produce emulsifying lipids. Lipase was mostly used for flavor improvement in bakery by releasing fatty acid short chains through esterification. In addition to the enhancement of flavor it has also been observed to modify the natural lipids in flour to strengthen the dough and prolong the shelf life of bakery products (Sachan & Singh, 2015). In *A. oryzae*, an artificially imparted lipase has been used as a processing succour in baking industries. Lipase, including all the hydrolytic enzymes, has been found to effectively reduce the initial firmness and increase the specific volume of slices of bread (Keskin et al., 2004). The increment in butter flavor for baked goods has been produced by butterfat hydrolysis with suitable lipase.

### ***Meat and Fish Processing and Food Dressing***

In meat and fish processing, lipase is utilized for removing fat and adding flavor. Lipase is found in fats and oils, which leads to fat breakdown into free fatty acids and glycerol. Lipase is also present in meat fat, eggs, fish, cereals, and milk. Lipase is widely used in whipping and mayonnaise dressing for texture and quality improvement (Sachan & Singh, 2015).

### ***Lipases in Tea Processing***

Lipase is extensively used in the processing of tea. The black tea quality largely depends on, enzymatic fermentation, dehydration, and mechanical braking. During black tea processing, the enzymic hydrolysis of lipids of the membrane commences volatile product formation with distinctive flavor attributes (Verma et al., 2012).

### ***Lipase in Oil and Fat Processing***

Oil and fats are important food components and their modification is one of the important areas in the food processing industries that require green and economic technologies. Lipases play a key role in modifying lipid properties by changing the fatty acid chain location in the glyceride and substituting them with other ones. Esterification and interesterification are being employed for obtaining products of high value through the lipolytic conversion of fats and oils (Rai et al., 2018). A research study has made an immobilized lipase membrane reactor for the hydrolysis



of fat and oil, produced products requiring minimized downstream processing and reduced the total cost of processing. The removal of phospholipids in vegetable oils, known as de-gumming, has also been developed as an eco-friendly process (Clausen, 2001). Triacylglycerol lipase generated from genetically modified *A. oryzae* has been used for oil de-gumming and to enhance the emulsifying properties. A new process for lipase immobilization based on silica granulation has been observed to simplify the process and reduce the processing cost. These methods have been widely applied for oil and fat production free from trans-fatty acids (Christensen et al., 2001).

### ***Lipase as a Biosensor in the Food Industry***

Immobilized lipase is effectively used as a sensor to quantitatively determine triacylglycerol as they are fast, accurate, and cost-efficient. The application of such lipase is important in food industries. The primary use of lipase as a biosensor is liberating glycerol from the triacylglycerol analytically and quantifying the released glycerol by an enzymatic or chemical method. An experimental study has developed a method for determining organophosphorus pesticides with a surface acoustic wave impedance sensor by lipase hydrolysis. Lipase immobilization is done on oxygen electrodes combined with glucose oxidase, which is thereby used as a lipid biosensor and tends to be used in determining cholesterol levels in blood and triglycerides (Hasan et al., 2006).

### ***Dietetics***

With the increase in risks involved in high intake of fat, an increasing demand for low-calorie fats and substitutes of fat is highly considerable, but should not be vulnerable to high ranges of temperature. The less caloric fats and substitutes of fats do not possess natural fatty acids but may be in line with the function and chemistry of natural fats resulting in the formation of such products that are deficient in essential fatty acids (EFA). The analysis of the tri glycerols has shown an increasing preference for the action of lipase for the primary positions in comparison to the secondary positions. The targeted tri glycerols should be useful both in the formulation of food products for infants and in applications of parental nutrition. The utilization of papain of a good quality enriched with lipase ensures fathomable approval of such products. Lipids containing oleic, palmitic, linoleic, and stearic acid, similar to human milk fat, have been produced by enzymic hydrolysis between fatty acids, stearic acid, tripalmitin, and hazelnut oil. For both stearic and oleic acids, the level of incorporation is increased with the time of reaction. The structured lipids produced have been observed to be used potentially in infant formulae (Sahin et al., 2005). It has also been stated that industry and academia collaboration would hasten the enzymatic processes at as successful commercial level (Undurraga et al., 2001).

## *Miscellaneous*

Lipase enzymes have been usually utilized in producing various products, starting from fruit juices to fermented vegetables. Lipase facilitates fat removal from fish and meat products (Sharma et al., 2001). It has been reported that lipase catalyzes and synthesizes sugar fatty acid esters. An experimental finding suggests that the supplementation of lipase in noodles has resulted in significant and soft textural attributes in noodles even though they have relatively low concentrations of acylglycerols in the formulations (Undurraga et al., 2001). In confectionery processing, lipase has been used in producing fat of high concentrations of 1,3 stearoyl-2-monoolein which could be also used as a substitute for shea stearine in the synthesis of equivalents of cocoa butter. Fats inhibiting the formation of bloom in chocolate products have also been synthesized by these types of enzyme esterification mechanisms (Macrae, 2000). *C. rugosa* lipases are widely used in the flavor and food industry, in single-cell protein and ice cream production, in the biocatalytic resolution of pharmaceuticals, in esters of carbohydrates, and in amino acid derivatives that are not obtained conventionally.

Immobilized lipase produced from *C. antarctica* has been used in the esterification of bioactive compounds along with fatty acids. Vitamins and secondary metabolites like kojic acid derived from microorganisms and plants can be acylated to form such products that are beneficial in the cosmetic, pharmaceutical, and food industries. Regioselective modification of polyfunctional organic compounds has been proved as an extended area of the application of lipase. The enzyme is also conjugated with a mixture of microbes for treating effluents enriched with fat derived from ice cream. It might also be used in the waste processing from the food industry.

## **Conclusion**

The applications of biotechnology in the production and processing of food encompass a very large and diverse field. Modern biotechnology is applied in the improvement of food taste, and yield, increasing nutritive values, and shelf life. It is also applied in fermentation and enzymic processes. Therefore, biotechnology can be employed for human health benefits, and eradicate malnutrition, and health ailments in developing countries. The  $\beta$ -galactosidase emerges as more promising in fruit juice preparation and breakdown of plant polysaccharides containing galactose and other hexoses. Genetically modified food technology is amongst the advanced technologies of the present era that would potentially combat hunger, malnutrition, and poverty. However, genetically modified food is opposed by a multitude of people. Various seminars should be conducted to make people aware of the potential pros and cons. Biotechnology should be introduced as an individual subject at the high school level to educate students about its benefits. It can potentially help in

combating many nutrition-related problems in developing countries. Various bioprocesses have been formed involving yeast as a single or a mixed starter along with filamentous fungi and lactic acid bacteria for nutraceutical production. With an increasing focus on yeast participation in the synthesis of nutraceuticals, the selection of potential yeast strains for the enhancement of the functional characteristics of the product has become an integral approach. Recent measures of biotechnology have also been employed to result in recombinant yeast production with enhanced characteristics for producing nutraceuticals. The potential use of lipase enzyme in the food industry demands to development of cost-efficient technologies for high production, scale-up, and purification of this potential enzyme. A myriad of hydrolytic applications, like the flavor development in butter, margarine, cheese, and milk chocolates, is a desirable area of the enzyme lipase. However, the new applications of lipases are yet to be examined in food industries. The lipase properties have been enhanced by genetic engineering to extend its applications in unfavourable conditions. A variety of changes in enzyme immobilization plays a key role in applying lipase as a biocatalyst in food processing and technology.

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