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Review

Fungal biotechnology in food and feed processing

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A B S T R A C T

Fungi are of excellent value nutritionally, and of great importance to vegetarians. Edible mushrooms are excellent sources of protein, have low-fat content and are free of cholesterol. They are easily cultivable and are consumed either in fresh or processed form. Yeasts and filamentous fungi secrete a plethora of important enzymes in the growth medium together with other secondary metabolites. Most of these are hydrolytic in nature being employed in different food processing industries as well as in refinement of fodder quality. Edible filamentous fungi producing these enzymes present an added advantage for their use in food and feed. In this article these aspects will be discussed along with the results from edible mushroom *Termitomyces clypeatus*, producing a wide variety of hydrolytic enzymes and products, from our laboratory. It is likely that the functional understanding of different enzyme classes will provide new applications within the food industry in the future.

Contents

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1. Introduction

The fungi as food and feed are very nutritive since they contain essential and nonessential amino acids. The use of fungi as dietary sources and in fermented beverages is in vogue since prebiblical times. Archaeological evidence traces association of edible wild mushroom to the inhabitants of Chile, almost 13,000 years ago (Rojas & Mansur, 1995); However, it was in China where the consumption of wild fungi was first reliably noted, several hundred years before the birth of Christ (Aaronson, 2000). The first records of fermentation and the use of fermented foods were found in Sumeria and Babylon (Elander & Lowe, 1994). Inspite of this age-old practice of consumption of fungi and their utilization in production of other food materials, their full potential was not explored until the later half of the 20th century when it was boosted by the advent of the golden age of industrial microbiology. Since then, this diverse community represented by yeasts, mushrooms and filamentous fungi have been exploited in a myriad of food products both for human and livestock consumption.

The fungal kingdom possesses certain natural advantages in terms of their dietary supremacy over the rest of the vegetarian platter. These are: (a) a good protein content (20–30% of dry matter) having all the essential amino acids (yeasts are especially enriched in lysine) thus capable of substituting meat, (b) chitinous wall to act as a source of dietary fibre, (c) high vitamin B content, (d) low in fat and (e) virtually free of cholesterol. The lucrativeness of mushroom cultivation is further enhanced by their low cost of production, since most of them can be cultivated on agro wastes or other industrial waste products. All agricultural production generates enormous waste because so little of each crop is actually used (5% in palm and coconut plantation, 2% in Sisal plantation, 7% in sugarcane plantation etc.). These can easily be handled by cultivation of mushrooms. For example, Oyster mushroom species (*Pleurotus ostreatus*, *Pleurotus cystidiosus*, *Pleurotus sajor-caju*) grows readily on cotton wastes. Similarly, although the straw mushroom *(Volvariella volvacea)* is traditionally grown in South-East Asia on rice straw, it too can be grown on cotton waste. The ability of certain *Pleurotus* sp. to grow on many lignocellulose agricultural wastes has been exploited both in bioremediation and production of a consequent cash crop in the form of mushroom.

Moreover, the harvested mushrooms (spent compost) can be used as a useful animal feed because of its high protein content, soil conditioner with its high content nutrients and polymeric components that enhance soil structure and even used to digest pollutants (like polychlorinated phenols) on land-fill waste sites by virtue of its population of microorganisms able to digest the natural phenolic components of lignin (Chiu, Ching, Fong, & Moore, 1998). Also, the multi-species amenability and subsequent ease of upgradation to large-scale cultures have substantially aided the use of fungi commercially. These factors have contributed in bringing together a number of research groups and companies who have participated in consecutive 'Eurofung' projects funded by the European Union to accelerate the growth of the industry. As a consequence, the annual average yield of mushrooms has climbed up to a staggering 6161 thousand tonnes (Carlile, Watkinson, & Gooday, 2001) and the trade has flourished as an intercontinental one. However, not all of the wild varieties are propagated on a commercial scale. The choice of the strain is made on the basis of production yields and regulatory issues, especially for fungi used in the food industry. Host strains are usually chosen from among those, which have attained the so-called GRAS (Generally Recognized As Safe) status, by the U.S. Food and Drug Administration (FDA).

In this article, the major applications of the fungal kingdom (namely macrofungi/mushroom, filamentous fungi and yeast) in food and animal fodder has been reviewed chiefly under two broad categories of (A) direct use of either fresh fruiting body or processed mycelia and (B) a more indirect fermentation based approach where the fungal enzymes and secondary metabolites have been employed in the processing of a wide range of food and health products. Still other important aspects of study may relate to the diversified applications of these organisms in nutraceuticals and pharmaceutical industries. However, to accommodate for the limitations in volume of the text, those issues have not been incorporated in detail. Finally the genus *Termitomyces* and *T. clypeatus* related to our own activity in this respect are mentioned.

2. Use of fungi in dietary food

Over recent years, consumption of fungal food had increased on a global basis with rise in public concern about dietary and health issues. Especially, those belonging to the vegetarian community have resorted to eating either freshly cooked mushrooms or processed foods, beverages and dietary supplements of fungal origin.

3. Use of fruiting body

Fruiting body of mushrooms have been consumed directly fresh or processed and used as delicacy. Fungi can be produced technically through fermentative process, through stages of media preparation, inoculation and incubation. The media may be in form of substrates available from cheap valued sources like agro-biomass and industrial waste; though transformed into high value added food and pharmaceutical products. Thus use of fungi is important from economical as well as environmental aspects.

Although many hundreds of species of edible mushrooms exist in the wild, less than 20 species are used extensively as food and only 8–10 species are regularly cultivated to any significant extent. The most commonly eaten species, *Agaricus bisporus,* sold as button mushrooms when small or Portobello mushrooms when larger, used in salads, soups, and many other dishes. Many Asian fungi are now commercially grown and have gained huge popularity in the West. They can be availed fresh from grocery stores and markets, including straw mushrooms (*Volvariella volvacea*), oyster mushrooms (*Pleurotus ostreatus*), shiitakes (*Lentinula edodes*), and enokitake (*Flammulina* spp.). They are often used for the preparation of various types of dishes. There are many other fungi like milk mushrooms, morels, chanterelles, truffles, black trumpets, and *porcini* mushrooms (*Boletus edulis*) (also known as 'king boletes'), all of them have a very high market price. The most commonly used cultivated edible macrofungi are enlisted in Table 1, along with their nutritional and known medicinal properties. They can be used as a vegetarian substitute of protein source with non-vegetarian sources. Fruiting body of fungi can be cultivated through horticulture.

'Shiitake Mushroom' (*Lentimula edodes*) is the second most cultivated mushroom in the world, only after *Agaricus*, the 'Paris mushroom'. Besides China and Japan, Shiitake is also widely cultivated in Taiwan, Thailand, Korea, Singapore as well as Holland, the United States and Canada. Shiitake's protein has a full complement of

Table 1
Some medicinal and nutritional properties of commonly used edible macrofungi.

Fungi (Common name)	Appearance	Nutritional properties	Medicinal properties	Reference
Lentimula edodes (Shiitake or shiang-gu)	Dark brown cap with white stalk.	Contains high protein with all essential amino acids; well known natural source of vitamin D; adenine and choline content effective in preventing the occurrence of cirrhosis of the liver as well as vascular sclerosis.	Tyrosinase contained in *Lentinula edodes* tends to lower blood pressure. Lentinam, an active polysaccharide a (1–3) β-D-glucan reduces cancer and cholesterol and enhances TH1 response	Murata, Shimamura, Tagami, Takatsuki, and Hamuro (2002), Rossi et al. (1993)
Volvariella volvacea (Straw mushroom)	Pink gills and spore prints; lack a ring; have an Amanita-like volva at the stem base. The gills of young Volvariella are white.	A natural source of antioxidant due to high β-carotene content	Contains a fungal immunomodulatory protein FIP- Vvo that induces TH1-specific cytokines (IL-2, IFN-γ, LT), TH2-specific cytokine (IL-4).	Hsu, Kao, Ko, Lin, and Lin (1997), She, Ng, and Liu (1998), Cheung, Cheung, and Ooi (2003)
Flammulina velutipes (Winter mushroom)	Convex shaped cap; moist and sticky when fresh; colour variable–dark orange brown to yellowish brown; Gills attached to the stem and whitish to pale yellow, becomes dark rusty brown on maturity.	Mannofucogalactan a heterogalactan derived from *Flammulina* is known to posses nutritional values	Induces antibody production by modulation of TH-cell Differentiation and function.	Ko, Hsu, Lin, Kao, and Lin (1995), Carbonero, Gorin, Iacomini, Sassaki, and Smiderle (2008), Smiderle et al. (2008)
Pleurotus ostreaus (Oyster mushroom)	White, gray-brown or ivory coloured and resembles oyster shell like shape. The white gills run down its short, off-centered white stalk.	Unique flavor and aromatic properties; considered to be rich in protein, fiber, carbohydrates, vitamins and minerals. Among the volatile compounds that constitute edible mushroom flavor, 1-octen-3-ol is considered to be the major contributor.	Promising as medicinal mushrooms, exhibiting hematological, antiviral, antitumor, antibiotic, antibacterial, hypocholesterolic and immunomodulation activities.	Cohen, Persky, and Hadar (2002)
Tuber melanosporum (Truffle)	Fruiting body or truffle is round, pitted and white when young but darkens as it matures.	Truffle has tantalizing taste and aroma and is most sought after delicacy with great economic value.	Regarded as therapeutic food having anti carcinogenic, anti cholesterolaemic and anti viral properties and also prophylactic properties with regards to coronary heart disease and hypertension.	Breene (1990)
Ganoderma lucidum (Reishi)	Large, hard and leathery fungus with sessile or stalked basidiocarps having tiny pores undersurface	Used in dietary preparation and to make tea or soup. Protein comprises only 7.3% of dry weight. Glucose accounted for 11% and metals 10.2% of dry mass (K, Mg, Ge and Ca being the major trace components)	GLIS, a proteoglycan isolated from the fruiting body is a B cell stimulating factor. This compound stimulates B lymphocyte activation, proliferation, differentiation and production of immunoglobulins	Bao, Wang, Dong, Fang, and LI (2002), Zhang, Tang, Zimmerman-Kordmann, Reutter, and Fan (2002)
Auricularia polytricha (Wood ear)	Ear shaped structure of fruiting body	Rich in P, Mg, K and Se; high dietary fiber content more than 50% of net weight. Helps in relieving constipation.	The fruiting body produces a new immunomodulatory protein (APP) enhancing the production of both nitric oxide (NO) and tumor necrosis factor-α (TNF-α), suggesting that APP is an immune stimulant and can increase the immune response of its host. APP activates murine splenocytes, markedly increasing their proliferation and gamma-interferon (IFNγ) secretion.	Sheu, Chien, Chien, Chen, and Chin (2004), Kim, Park, Choi, Lee, and Kim (2004).
Tremella fuciformis (Silver ear)	Gelatinous, jelly-like basidiocarps having leaf–like folds.	Widely eaten in the east; high fiber content makes it popular among dieters and cholesterol affected people.	Very high dietary fiber content, have potential hypocholesterolemic effect, similar to other high fiber foods.	Cheung (1996).
Morchella esculenta (The common morel)	White ridges and dark brown pits; with age both ridges and pits turn yellow	Morels are a feature of many cuisines including Provencal.	Methanolic extracts from *Morchella esculenta* include antioxidant activity, reducing power, scavenging effects on radicals, and chelating effects on ferrous ions; contains galactomannan that induces macrophage activity.	Duncan, Pasco, Pugh, and Ross (2002)
Morchella elata (The black morel)	Ridges are gray or tan; pits are brown and elongated when young; turn black with age.	Rich in Vitamin D2.	Chinese believe it can cure tuberculosis, high blood pressure and common cold.	Mattila, Piironen, and Suonpaa (2000)
Morchella semilibera (The half-free morel)	Small caps and long bulbous stems. The bottom of the cap is attached directly to the stem.	Spongy texture of young morels makes delicious dishes.	The ethanolic extract of Morchella has 85% of antioxidant property.	Carbonero et al. (2008)
Agaricus bisporus (Button mushroom, Champignon)	The original wild form bear a brownish cap and dark brown gills but more familiar ones are with a white cap, stalk and flesh and brown gills.	Fairly rich in vitamins like vitamin B and minerals like sodium, potassium, phosphorus and selenium. Raw mushrooms are naturally cholesterol and fat free.	Effective in reducing blood serum cholesterol; good dietary source of B-complex vitamins especially riboflavin,	Beelman, Royse, and Chikthimmah (2003)

essential amino acids so it can be used extensively in a vegetarian diet. Its active ingredient, Lentinan (a polysaccharide), has been shown to reduce cancer and cholesterol. The 'Shiitake mushroom' is as common in Asian countries as *Agaricus bisporus* is in the west. Its cultivation method is similar to that of *P. ostreatus*. *Volvariella volvacea* also known as 'paddy mushroom' and is incorporated in many Chinese recipes. It is commercially cultivated on a mixture of raw cotton waste and rice bran and harvested in the button or egg stage before the pileus emerges. In the wild, the fungus tends to grow on decaying vegetation and wood. 'Winter mushroom' or 'Enoki' (*Flammulina velutipes*) is a very small and tender mushroom. It is cultivated on sawdust medium in a large container. It would seem to be an unlikely candidate for cultivation because of its small size, but commonly sold in supermarkets. The origin of cultivation of this species is believed to be in Japan. 'Oyster mushroom' (*Pleurotus ostreatus*) is a saprobic fungus that can commonly be found, growing on dead trees, in nature. 'Truffle' (*Tuber melanosporum*), a fungus belonging to the order Tuberales, is a subterranean European fungus and has been collected since at least 3600 years. The flesh of all truffles is nearly white when young; as the truffle matures the flesh becomes darker with a marbling of lighter tissue. The taste and aroma of commercially collected truffles is so intense that they are used as a flavoring instead of a separate dish. Another mushroom called 'Lingzhi' or 'Reishi' (*Ganoderma lucidum*) is not quite a palatable mushroom, however used as consumable in dietary preparations, but is incorporated as one of the most respected ingredients in traditional oriental medicine. It is cultivated for its medicinal and tonic values. The earliest record of the species *Auricularia polytricha* known as 'wood ear' dated back to about 200–300 BC. It is now cultivated throughout the South Pacific and Asia. It is referred as Mu-Er (wood ear) in China and Pepiao (ear) in Hawaii. The cultivation of these species is the same as that of the Shiitake Mushroom. It is cultivated on logs and also on a mixture of sawdust and cotton waste. Commonly known as 'Jelly Fungi', because of the gelatinous jelly-like nature of the basidiocarps, which consist of leaf-like folds, *Tremella fuciformis* is also known as 'silver ear' or 'snow ear' fungus, is widely eaten in the east and is regarded as Chinese delicacy. It has been long utilized as a "herb" to cure many ailments. Chinese believe that it could cure tuberculosis, high blood pressure and common cold. The method of cultivation of this species is identical to that of the *Shiitake* and *Auricularia* since it is a wood inhabiting species. True *Morels*, is a genus of edible mushrooms closely related to anatomically simpler cup fungi. Though morels are typically sold dried or canned, they can be purchased fresh. When preparing fresh morels for consumption, soaking them may ruin their delicate flavor. Due to their natural porousness, morels may contain trace amounts of soil which cannot be washed out. One of the best and simplest ways to enjoy morels is by gently sauteeing them in butter, cracking pepper on top and sprinkling with salt. Morels are popular in different varieties: *Morchella esculenta* better known as 'The common morel' When young, this species has white ridges and dark brown pits and is known as the "white morel." As it ages, both the ridges and the pits turn yellowish brown, and it becomes a "yellow morel".

4. Fungi as and in processed food

Fungi constitute a fair share of food and food additives in the markets as animal feed or human food.

4.1. SCP

Single Cell Protein is the name collectively given to a variety of microbial products, produced by fermentation. They can be used to ferment some of the vast amounts of waste materials, such as straws; wood and wood processing wastes; food, cannery and food processing wastes; and residues from alcohol production or from human and animal excreta. These factors collectively may see SCP emerge as the potential protein source for domestic livestock. Generally, they are obtained in a diluted form containing less than 5% solids, which are further concentrated by methods like filtration, precipitation, coagulation, and centrifugation. Removal of water is necessary to stabilize the material for storage in most instances; Single cell protein must be dried to about 10% moisture, or condensed and acidified to prevent spoilage from occurring, or fed shortly after being produced; however, these dehydration methods are not currently economical. Another important aspect of exploitation is the production of protein from hydrocarbon wastes of the petroleum industry by employing filamentous fungi. These organisms have ease of harvesting but suffer from slow growth rates. In comparison, yeasts have better production and utility values and as such have established their dominance over other members of the community. The major uses of yeasts have been highlighted as follows.

4.2. Baker's yeast

The production of baker's yeast is the largest domestic use of a microorganism for food purposes. Baker's yeast is a strain of *Saccharomyces cerevisiae.* The strain of the yeast is carefully selected for its capacity to produce abundant gas quickly, its viability during ordinary storage, and its ability to produce desirable flavour. The organisms are mixed with bread dough to bring about vigorous sugar fermentation. The carbon dioxide produced during the fermentation is responsible for leavening or rising of the dough.

4.3. Use of yeast cells in food and fodder

The terms 'food yeast' or 'fodder yeast' are confined to a particular type of single cell biomass. Professor Jacquot and Dr. Biloraud in 1957 first proposed the definition of food yeast, which was subsequently adopted by the I.U.P.A.C. and then by the European Economic Community in 1975. The version goes as "Food yeast is a yeast that has been killed and dried; it should have no diastase activity, and has not been submitted to extraction process nor received any additive." Yeasts acting as food supplements of domestic livestock can be safely termed as fodder yeasts. Essentially, these organisms intended for human and animal consumption must comply with certain nutritional properties of vitamin content, protein content, amino acid composition, good digestibility, and absence of toxic substances.

In addition to these basic criteria, the strains chosen should be easily accomodable to large-scale production and should be cost effective to compete at par with other more conventional products in the market. The following aspects must therefore be clearly defined for a given strain used to produce food and fodder yeast, the growth conditions, the choice of potential substrates, and the associated downstream treatments.

5. Processed fungal food as an alternative to SCPs

Other than these, several popular processed food products are available in the market the most notable of which is the myco-protein 'Quorn'. Myco-protein the term coined by the UK Foods Standards Committee to serve as the generic name for a food product resulting from the continuous fermentation of a selected strain of *Fusarium venenatum* (originally called *F. graminearum*). Originally the product was dried and powdered for sale as high protein SCP flour but for its organoleptic qualities of the hyphal mass it has been developed as a meat substitute under the brand name 'Quorn' as a high-technology product. The mycelium is grown in a very large air-lift fermenter in a continuous-culture mode. Its filamentous structure enables it to simulate the fibrous nature of meat; coupled with the inherent nutritional value of fungal biomass, the product is a low-fat, low-calorie, cholesterol-free health food.

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5.1. Use in fermentation based food industries

Although, the cultivation of macrofungi had flourished in recent years, use of fresh mycelia as food has not still attained the impetus to be popularized on a global scale. Rather, more indispensable is the role of these organisms especially yeast in industrial food production and processing. Fungal cell factories are widely employed in brewing, wine making, and bread making industries due to their inherent capacity of secretion of a wide titer of enzymes into the growth medium. The following sections will introduce and elaborate these indirect but vital aspects of fungal usage.

5.2. Production of alcoholic beverages

The yeast *Saccharomyces cerevisae* is widely used for the production of many alcoholic beverages. They have been classified into three categories; those produced using fruit juices, those produced using starchy materials, and those produced using other plant materials (Carlile et al., 2001). The limitations imposed by alcohol concentration on the fermentation by yeast is in the ranges of 10–12% or 15–16% as in the case of 'sake' production and the alcoholic concentrations is increased by distillation. Alcoholic beverages produced using fruit juices include wine, cider, and perry. Wines are produced by fermenting red and white grapes, where the yeast converts the fruit sugars into alcohol. *Botrytis cinerea*, a grape vine pathogen, is used to produce a particular wine "Sauternes" or "Edelfaule" in Germany. The pathogen attacks the growing grapes and concentrates the sugars inside and removes any residual acidity, the grapes are then harvested and fermented using the traditional methods. The resulting wine is very sweet and of high quality. Cider is produced using apples. Ciders can be classified into sweet (low in acid and tannin), bittersweet (low in acid, high in tannin), sharp (high acid, low tannin), and bitterhsharp (high in both acid and tannin). There is less sugar in apples than grapes decreasing the alcohol concentration of cider. Perry is produced using Perry pears or dessert pears. Again, the same fermentation process as used for wine and cider is used for the production of Perry.

Regarding alcoholic beverages produced from starchy materials, the most widely used material is cereal grain (rye, barley, wheat). However, other starchy materials are also used such as seeds, roots, and tubers. Before fermentation proceeds, the starchy material has to be degraded into simpler sugars. In the beer industry, malting is used to achieve that goal. Malting occurs under aerobic conditions in which typically barley grains are steeped in water for up to a day and then allowed to germinate under moist aerobic conditions. The germination process produces enzymes, which degrade the polysaccharides and proteins into simpler sugars and amino acids. The material is fermented into beer. In Japan, the production of 'sake' uses rice as their starting material. Rice is rich in starch and thus unutilisable by the yeast. Therefore, *Aspergillus oryzae*, a fungus is inoculated to grow on the surface of the rice. The fungus converts the starch into simpler sugars, which then can be used by the yeast to produce sake. 'Sake' is inoculated with *Aspergillus oryzae* to produce 'koji'. Further rice mash is lactic acid fermented using bacteria and yeasts. The mash and 'koji' are mixed and fermented for around 20 days, till the alcohol concentration has reached around 18%. The product is filtered, pasteurized and stored before consumption.

Production of non-alcoholic beverages like 'Coco' (*Leuconostoc mesenteroides, Candida* sp.), Coffee (*Leuconostoc mesenteroides, Saccharomyces marscianus,Flavobacterium* spp., *Fusarium* spp.) (Soccol et al., 2008), Black Tea (Pasha & Reddy, 2005).

5.3. Production of bakery and cheese products

Bakery products consist of a mixture of flour (usually from cereals especially wheat), with water, salt and sugar, leavened by yeast. Flour is mixed with the remaining ingredients and incubated at about 25 °C. The yeast ferments the sugar forming carbon dioxide and alcohol. The released gas causes bubbles by elastic extension of gluten (a protein) in the flour. On baking, the alcohol evaporates. The length of leavening, the quantity of gluten in the flour, the constituents of the grain and the temperature determine the texture and flavor of the bread.

In production of cheese presence of visible fungal mycelium is a part of the moldy cheeses favorites among gourmets. Two of the most familiar examples are 'Camembert' and 'Roquefort', also known as 'blue cheese'. These cheeses are made from two species of *Penicillium*, *P. camemberti*, in Camembert cheese and *P. roqueforti* in Roquefort cheese. Among one of the earliest of manufactured foods, the discovery of the cheese making process, are in use since approximately 4000 years ago, and then molds and other microorganisms were added to cheese for flavor. The processes are usually referred to as mold-ripened.

5.4. Production of other food products/condiments/additives

A great deal of research has been carried out on some of the fermented food products, so that the identity of the fungus involved in the process has been established. Some of the more familiar ones include 'miso', 'shoyu', 'tofu' and 'tempeh'. However, the microorganisms (including bacteria) involved in the majority of fermented food (approximately 500 in numbers) are unknown. Unlike western cultures, in which fermented food is usually carried out by yeasts, eastern cultures have utilized a number of different mycelial fungi. 'Soya Sauce' (Shoyu or soy sauce) is one of the most familiar asian food products made by cooked soybeans mixed with wheat flour, pressed into cakes and inoculated with *Aspergillus oryzae*. The molded cake known as 'Koji', mixed with salt and water is referred to as the 'Moromi'. The moromi is then inoculated with a bacterium, *Peiococcus soyae,* and yeasts *Saccharomyces rouxii* and *Torulopsis* sp., to ferment the mixture for approximately 6 months to turn into the soy sauce. Shoyu, discovered in China more than 2500 years ago, is known in the West as a flavoring and flavor-enhancing ingredient as meatless seasoning. 'Tempeh' made from the fermented products of legume seeds with *Rhizopus oligosporus*, is believed to have originated in Indonesia. The inoculation of the fungus into the boiled beans digests the complex carbohydrates and other organic compounds that may cause gas. 'Miso' is a japanese word for fermented soybean paste, not usually consumed by itself, but as a base for soup or used as a flavoring agent. 'Miso' fermentation consists of washed, polished rice, which is steamed and inoculated with *Aspergillus oryzae* resulting in 'rice koji'. The carbohydrates and proteins of the inoculated rice are digested by the fungus and converted to sugars and amino acids. The 'rice koji' is then inoculated by yeasts and bacteria and allowed to ferment.

All of the above processes are actually carried out by a host of fungal enzymes, which are released into the respective substrates by the concerned fungi. These enzymes with their brief area of application in the food processing industries are listed in Tables 2 and 3.

6. Use of enzymes in food and feed bioprocessing

Enzymes have been used in food manufacturing since the dawn of mankind in cheese manufacturing and indirectly via yeasts (Schmid et al., 2001). In food industry in addition to cheese manufacturing enzymes were used already in 1930 in fruit juice

Table 2
Enzymes associated with food and feed bioprocessing.

Industries	Enzymes	References
Butter and butter oils	Catalase, glucose oxidase, lipase	Gupta, Rathi, and Bradoo (2003)
Cheese	Rennet, lipase, proteinases	Freitas and Malcata (2000)
Animal Feed	Amylase, glucoamylases, glucanase, cellulases, pentosanases, xylanases, proteinases, phytases	Wang, Bai, and Liang (2006)
Alcohol	Amylase, amyloglucosidase, β-glucanases, cellulases, cellobiase, pectinase, proteinases	Sharma, Pandey, and Saharan (2002)
Biscuits	Amylases, cellulases, hemicellulases, proteinases, pentosanases	Taniwaki, Silva, Banhe, and Iamanaka (2001)
Breads	Amylases, amyloglucosidases, cellulases, glucanases, glucose oxidase, hemicellulases, lipases, pentosanases, proteinases	Taniwaki et al. (2001)
Brewing	Acetolactase, decarboxylase, amylases, amyloglucosidase, cellulase, glucanase, lipase, pentosanase, proteinase, xylanase	Okamura et al. (2001)
Coffee	Cellulase, hemicellulases, galactomannanase, pectinase	Soccol et al. (2008).
Confectionery	Amylase, invertase, pectinase, proteinase	Stroh (1998)
Egg processing	Proteinase, lipase phospholipase, Catalase, glucose oxidase	Singh, Wakeling, and Gamlath (2007)
Fats	Esterase, glucose oxidase, lipases	Bobek, Ozdin, and Kuniak (1994)
Fish	Proteinase	Prasad (2001)
Dairy products	Lactase, Proteinase, sulphydryl oxidase, lactoperoxidase, lysozyme, peroxidase, catalase	Archer (2000), Beauchemin, Yang, and Rode (1999), Rode, Yang, and Beauchemin (1999)
Dibittering	Peptidase, naringinase	Knoss et al. (1998)
Flavors	Glucanase, peptidase, proteinase, esterase, lipases, amylase	Shahani, Arnold, Kilara, and Dwivedi (1976)
Fructose	Glucose isomerase, inulinase, Amylase, amyloglucosidase, cellulase, glucanases, hemicellulases, isomerase, lipase, phospholipase, pectinases, proteases	Sørensen et al. (2004)
Fruit, cloudy juices	Amylases pectinases, cellulases, proteinase	Brandelli, Geimba, and Mantovani (2005)
Fruit extracts	Anthocyanase	Albershein (1966)
Vegetable and fruit Processing	Cellulases, macerating enzymes, pectinases	Brandelli et al. (2005)
tea	Cellulase, glucanase, pectinase, tannase	Pasha, and Reddy (2005)
Wine	Amylase, amyloglucosidase, cellulase, glucanase, hemicellulase, pectinases, proteases, glucose oxidase, catalase, pentosanase, anthocyanase	Okamura et al. (2001)
Malt extract	Amylase, amyloglucosidase, cellulase, glucanase, proteinase, xylanase	Feng, Larsen, and Schnürer (2007)
Botanical extraction	Amylase, amyloglucosidase, cellulase, glucanase, hemicellulase, pectinases, proteases	Knoss et al. (1998)
Animal oil/fats	Esterases, Lipases, proteinase	Beldman, Pilnik, Rombouts, and Voragen (1984)
Protein	Amylase, cellulase, glucanase, hemicellulase, pectinase, protease	Semenova et al. (2006)
Starch	Amylase, amyloglucosidase, cellulase, glucanase, hemicellulase, isomerase, lipase, phospholipase, pectinases, proteases	Albershein (1966), Ezeogu Okolo, Mba (1995)
Fruit extraction	Amylase, amyloglucosidase, cellulase, pectinase, pentosanase, limonoate, dehydrogenase, naringinase	Albershein (1966), Kashyap, Vohra, Chopra, and Tewari (2001)
Fruit juice	Amylase, amyloglucosidase, cellulase	Semenova et al. (2006)
Fruit pulps	Pectinase, amylase, amyloglucosidase, cellulase, glucanase. hemicellulase, pectinase, protease	Brandelli et al. (2005)

Table 3
Some common enzymes used as additives in food and feed.

Enzymes	Applications
α-Amylase[a]	Starch syrups, fermentation, ethanol, animal feed
β-Amylase[a]	Brewing, maltose syrup
Cellulase[a]	Animal feed
β-Glucanase	Brewing industry
β-Glucosidase[a]	Transforms isoflavone phytoestrogens in soymilk
Dextranase	Hydrolyzes the polysaccharide dextran
α-Galactosidase (melibiase)	Could increase yield of sucrose; potential use in the beet sugar industry
Glucoamylase[a]	Manufacture of dextrose syrup and high-fructose syrup
Hemicellulase/ Pentosanase/Xylanase[a]	Baking, fruit juice manufacture
Invertase[a]	Manufacture of invert syrup from cane or beet sugar
Lactase	Eliminates lactose from dairy foods
Pectinase[a]	Fruit processing
Naringinase	Debitter citrus peel
Pullulanase	Antistaling agent in baked goods
Proteases[a]	Brewing, baking goods, protein processing, distilled spirits

[a] All these enzymes are produced from *Termitomyces clypeatus*.

manufacturing. These enzymes clarify the juice. They are called pectinases, which contain numerous different enzyme activities. The major usage of microbial enzymes in food industry started in 1960s in starch industry. The traditional acid hydrolysis of starch was completely replaced by α-amylases and glucoamylases, which could convert starch with over 95%, yield to glucose. Starch industry became the second largest user of enzymes after detergent industry. Intensive study to use enzymes in animal feed started in early 1980s. The first commercial success was addition of β-glucanase into barley based feed diets.

Enzymes were tested later also in wheat-based diets. Xylanase enzymes were found to be the most effective ones in this case. Xylanases are nowadays routinely used in feed formulations. Usually a feed enzyme preparation is a multienzyme cocktail containing glucanases, xylanases, proteinases and amylases. Enzyme addition reduces viscosity, which increases absorption of nutrients, liberates nutrients either by hydrolysis of non-degradable fibres or by liberating nutrients blocked by these fibres, and reduces the amount of faeces. In addition to poultry, enzymes are used in pig feeds and turkey feeds. Enzymes also have many applications in drink industry. Chymosin is used in cheese making to coagulate milk protein. Another enzyme used in milk industry is β-galactosidase or lactase, which splits milk-sugar lactose into glucose and galactose. This process is used for milk products that are consumed by lactose intolerant consumers.

Enzymes are used also in fruit juice manufacturing. Addition of pectinase, xylanase and cellulase improve the liberation of the juice from the pulp. Pectinases and amylases are used in juice clarification. Brewing is an enzymatic process. Malting is a process, which increases the enzyme levels in the grain. In the mashing process the enzymes are liberated and they hydrolyse the starch into soluble fermentable sugars like maltose, which is a glucose disaccharide. Additional enzymes can be used to help the starch hydro-

Table 4
Recombinant enzymes from fungi.

Enzyme	Host	Donor
Catalase	*A. niger*	*Aspergillus* sp.
Cellulase	*A. oryzae*	*Humicola* sp.
Cellulase	*T. reesei* (*longibrachiatum*)	*Trichoderma* sp.
β-galactosidase	*A. oryzae*	*Aspergillus* sp.
β-glucanase	*T. reesei* (*longibrachiatum*)	*Trichoderma* sp.
Glucose oxidase	*A. niger*	*Aspergillus* sp.
Lipase	*A. oryzae*	*Candida* sp. *Rhizomucor* sp. *Thermomyces* sp.
Phytase	*A. niger, A. oryzae*	*Aspergillus* sp.
Xylanase	*A. niger* (and var. *awamor*), *A. oryzae, T. reesei* (*longibrachiatum*)	*Aspergillus* sp. *Aspergillus* sp. *Thermomyces* sp. *Trichoderma* sp.
Chymosin	*A. niger var. awamori*	Calf
Protease	*A. oryzae*	*Rhizomucor* sp.

References: (Archer (2000), Dunn-Coleman et al. (1991), and Pariza and Johnson (2001)).

lysis (typically α-amylases), solve filtration problems caused by β-glucans present in malt (β-glucanases), hydrolyse proteins (neutral proteinase), and control haze during maturation, filtration and storage (papain, α-amylase and β-glucanase). Similarly enzymes are widely used in wine production to obtain a better flavor and colour.

7. Fungal enzymes used in feed

Enzymes have been used for decades to improve the utilization of swine and poultry diets. Several recent studies have examined the use of exogenous enzyme products in high-forage diets fed to growing cattle (Beauchemin, Jones, Rode, & Sewalt, 1997; Beauchemin, Rode, & Sewalt, 1995; McAllister et al., 1999; Michal, Johnson, & Treacher, 1996; Pritchard, Hunt, Allen, & Treacher, 1996; Wang et al., 1999; ZoBell, Weidmeier, Olson, & Treacher, 2000) (Table 2). For instance phytase, amylase, β-glucanase and xylanase are added to the cereal-based diets of such monogastrics to increase the utilization of dietary phosphorous, starch, β-glucans and arabinoxylans, respectively. Research has demonstrated that supplementing dairy cow and feedlot cattle diets with fiber degrading enzymes has significant potential to improve feed utilization and animal performance (Beauchemin & Rode, 1996) (Table 3). Ruminant feed enzyme additives, primarily xylanases and cellulases, are concentrated extracts resulting from bacterial or fungal fermentations that have specific enzymatic activities. Improvements in animal performance due to the use of enzyme additives can be attributed mainly to improvements in ruminal fiber digestion (Arambel, Weidmeier, & Walters, 1987) resulting in increased digestible energy intake. This approach offers exciting possibilities for using enzymes to improve nutrient digestion, utilization, and animal productivity and at the same time reduce animal fecal material and pollution. Spraying enzymes onto feeds just before feeding provides increased management flexibility and bypasses any negative interactions that the ensiling process may have on silage enzyme performance. Treating feeds with enzymes in this manner may improve digestibility via a number of different mechanisms including, direct hydrolysis, improvements in palatability, changes in gut viscosity, complementary actions with ruminal enzymes, and changes in the site of digestion.

Protease enzymes may improve the digestion of cereal grains, because starch digestion is partially a function of the protein–starch matrix within the seed. Treating steam flaked sorghum with an enzyme mixture improved weight gain and feed efficiency in steers by about 10% (Boyles, Richardson, Robinson, & Cobb, 1992). Fiber degrading enzymes may also help to improve the digestion of cereal grains with fibrous seed coats. Cellulase/xylanase enzymes sprayed onto a barley and barley silage diet improved weight gain and feed efficiency in steers (Beauchemin & Rode, 1996).

Fungal DFM (Direct-fed Microbials) have been popular additions to ruminant diets for many years. In general, three types of additives are available. First, some products contain and guarantee "live" yeast. Most of these products contain various strains of *Saccharomyces cerevisiae* (Martin & Nibs, 1992; Savage, 1987). Second, other additives contain *Saccharomyces cerevisiae* and cultures extracts, but make no guarantee for live organisms. Third, there are fungal additives based on *Aspergillus oryzae* (AO) fermentation end products that also make no claim for supplying live microbes. Direct-fed microbial products are available in a variety of forms including powders, pastes, boluses, and capsules. In some applications, DFM may be mixed with feed or administered in the drinking water.

8. Commercial recombinant enzymes from fungi

Recombinant fungi are one of the main sources of enzymes for industrial applications. The industrial enzyme market reached $1.6 billion in 1998 (Stroh, 1998) for the following application areas: food, 45%; detergents, 34%; textiles, 11%; leather, 3%; pulp and paper 1.2%. This does not include diagnostic and therapeutic enzymes. The market for these non-pharmaceutical proteins reached $2 billion in 2000. Over 60% of the enzymes used in the detergent, food, and starch processing industries are recombinant products (Cowan, 1996); although the number of heterologous fungal enzymes approved for food applications is not very large (Table 4). Due to the low yields achieved with non-fungal proteins, many recombinant food-grade proteins are of fungal origin (Archer, 2000). There is one exception in which the donor strain is not another fungus, i.e., calf rennin (chymosin), which is used for cheese making. Production of this bovine protein in recombinant *Aspergillus niger* var *awamori* amounted to about 1 g/l after nitrosoguanidine mutagenesis and selection for 2-deoxyglucose resistance (Dunn-Coleman et al., 1991) Further improvement was done by parasexual recombination, resulting in a strain producing 1.5 g/l from parents producing 1.2 g/l (Bodie, Armstrong, & Dunn-Coleman, 1994). A recombinant strain of *Aspergillus oryzae* producing an aspartic proteinase from *Rhizomucor miehei* has been approved by FDA for cheese production (Pariza & Johnson, 2001). Microbial lipases have a huge potential in areas such as food technology, biomedical sciences, detergent and chemical industries. In the food industry, lipases are commonly used in the production of fruit juices, baked foods, desirable flavors in cheeses, and interesterification of fats and oils to produce modified acylglycerols. There are three fungal recombinant lipases currently used in the food industry, those from *Rhizomucor miehei*, *Thermomyces lanuginosus* and *Fusarium oxysporum*, all of which are produced in *A. oryzae* (Pariza & Johnson, 2001). The application of hydrolytic lipases in laundry detergents is another major sector of commercial usage. Detergent enzymes make up nearly 32% of the total lipase sales.

Lipase for use in detergents needs to be thermostable and remain active in the alkaline environment of a typical machine wash. In 1995, two bacterial lipases were introduced—'Lumafast' from *Pseudomonas mendocina* and 'Lipomax' from *Pseudomonas alcaligenes* by Genencor International (Jaeger & Reetz, 1998) which were found to be suitable for the purpose. Biotechnology has good prospects to increase the quality and supply of feedstocks for pulp and paper, reduce manufacturing costs, and create novel high value products. Novel enzyme technologies can reduce environmental hazards and alter fiber properties. Wild and recombinant hydrolases and oxidoreductases possess high potential for eco-friendly paper pulp bleaching (from pulp). The final lignin content of the flax pulp is even more decreased if a peroxide stage is included. This property has been exploited by cloning these genes (feruloyl esterase from *Aspergillus niger*, Mn^{2+}-oxidizing peroxidases from *Phanerochaete chrysosporium* and *Pleurotus eryngii*) into a totally chlorine free (TCF) sequence that also included a peroxide stage (Sigoillot et al., 2005).

9. Secondary metabolites from fungi used in food and feed

Secondary metabolites are compounds produced by an organism that are not required for primary metabolic processes. Fungi produce an enormous array of secondary metabolites, some of which are important in industry. They are largely employed to enhance the colouration of the food products. Fungi produce a range of compounds that alter the colour of food. For instance, *Monoascus purpureus* has been traditionally used for the production of red wine since long time (Went, 1895). The pigments are polyketides that are insoluble in acid conditions. β-Carotene is produced by a range of Mucorales (Ende & Stegwee, 1971). This can be added to a variety of foods. Concern with the potentially toxic or allergic characteristics of some artificial colors has led to a closer examination of colours from natural sources Fermentation of *Monascus purpureus* on rice to prepare 'koji' or 'ang-kak' (red rice) has been used as a traditional Chinese food and medicine since 800 A.D (Li, Zhu, & Wang, 1998). The water-soluble red pigments monascorubramine and rubropunctamine are produced by reaction of the orange pigments monascorubrin and rubropunctatin with amino acids present in the fermentation media. The fungus is used for preparing red rice, wine, soy bean cheese, meat, and fish and is authorized for food use in China and Japan. The yeast *Phaffia rhodozyma* has become the most important microbial source for the production of the carotenoid astaxanthin. This pigment is responsible for the orange to pink colour of salmonid flesh and the reddish colour of boiled crustacean shells. Feeding of penreared salmonids with a diet containing this yeast induces pigmentation of the white muscle. *Blakeslea trispora* has been used for the industrial production of β-carotene in Russia for years (Nolis & De Leenheep, 2008). Fermentation, a fungal mated culture is used with a preferred ratio of minus and plus mating strains. The accumulation of β-carotene is strongly linked to sexual interaction between the two mating types. A hormone-like substance produced during mating, the major component of which is trisporic acid, stimulates pigment production.

A group of harmful secondary metabolites which often contaminates animal feed are the mycotoxins (Bennett & Klich, 2003). These compounds are endowed with toxic properties towards humans and other animals, causing a wide range of acute and chronic effects collectively known as mycotoxicoses. Blending of the contaminated feed with uncontaminated one is a common practice to reduce mycotoxin contamination. However, this is under stringent regulation by the federal agencies. U. S. Food and Drug Administration (FDA) generally establish limitations for concentrations of mycotoxins in animal and human foods. Limitations are labeled using different terms, including "action levels" for aflatoxins, "guidance levels" for fumonisins, and "advisory levels" for vomitoxin. The legalities of such designations and their application to animal production are beyond the scope of this review.

10. Pharmaceutical and nutraceutical byproducts from fungi

Mushrooms with medicinal impact are used for nutraceutical and pharmaceutical products (Kidd, 2000; Wasser & Weiss, 1999). Due to their high tolerance and compatibility with the chemotherapy and radiotherapy the products obtained from mushrooms are used for the cancer therapies. The fruit bodies of mushroom and their extracts are effectively used and are also economically feasible option due to faster growth of fruiting body or the mycelial stage. Few prominent mushrooms having pharmacological properties are *Agaricus brasiliensis, Ganoderma lucidum, Lentinula edodes, Coriolus versicolor, Pleurotus ostreatus, Grifola frondosa*, *Termitomyces*, etc. (Chu, Ho, & Chow, 2002; Rossi et al., 1993). The important bioactive substances found in the mushroom included lentinan, schizophyllan and PSK, all used in the cancer chemotherapy as component of the drug cocktails, with the impressive results. Other biologically active substances found in the mushrooms included immunosuppressive, nematicide, antimicrobial, antiviral, and hypocholesterolemic agents. Black tea fermented with *Dabaryomyces hansenii*, results in accumulation of major vitamins, such as A, B1, B2, B12 and C in sufficient quantities to fulfill the recommended dietary allowance (RDA). It also results in reduction of caffeine and tannins in significant amount. Moreover, the theophylline accumulated as a result of fermentation imparts a bronchodilatory effect to the tea (Pasha & Reddy, 2005).

11. Symbiotic fungus *Termitomyces* (a filamentous basidiomycota)

Termitomyces is a paleotropical genus of agarics intriguing both to mycologists and entomologists. *Termitomyces* only grow in association with termites and their nests and are dependent on the organic matter brought by the insects from their feeding on trees. Although *Termitomyces* are saprobic, they are symbiotic with termites and contain important wild edible species. The fungus helps the termites to degrade the plant-derived material (e.g., wood, dry grass, and leaf litter) on which they live. Twenty edible species of *Termitomyces* have been recorded from Africa and Asia. These fungi are regularly collected and also sold. *T. titanicus* is the world's largest edible fungus according to Guinness Book of Records) has a cap diameter around 1 m, although other species *Termitomyces microcarpus* (rarely exceeds 2 cm) are much smaller. (Bignell, 2000; Natarajan, 1977). Twenty-three edible species of *Termitomyces* are reported from 35 countries. The genus is highly esteemed and many species are widely eaten with high nutritional value. The mushrooms are collected throughout Africa and are used widely in Asia, but are not well documented. Notable species include *T. clypeatus, T. microporus* and *T. striatus*. The species has medicinal properties and regarded good for brain and memory (Wei & Yao, 2003).

12. *Termitomyces clypeatus*: an edible fungus and producer of enzymes

T. clypeatus contains 31% protein, 32% carbohydrate and 10–14% ascorbic acid (Ogundana & Fagade, 1982). Several enzymes of high therapeutic values have been reported from *T. clypeatus* (Rouland-Lefe'vre, 2000).

Termitomyces clypeatus is known as a potential producer of different enzymes in culture media (Khowala & Sengupta, 1992;

Khowala, Ghosh, & Sengupta, 1992). The fungus *Termitomyces clypeatus* has been found to be a potential producer of a broad spectrum of extracellular glucosidases (cellulase, sucrase, cellobiase etc.), capable of hydrolysing the polysaccharides, e.g., hemicellulose, cellulose, and starch. Different enzymes such as endo 1,4-β-D-Xylanase, 1,4-β-D-Xylosidase, α-L-arabinofuranosidase, acetyl esterase, α-amylase, and amyloglucosidase were also purified from the fungus. All these enzymes have various industrial applications. Few other enzymes studied from this fungus are enlisted along with their use (Table 3).

13. Bioprocessing of food by *T. clypeatus*

13.1. Softening and leavening of bread

A process for the preparation of a novel enzymatic formulation useful for improved leavening of bakery products was developed from *Termitomyces clypeatus* (Sengupta, Ghosh, Naskar, Sengupta, & Jana, 1999). The enzymes hemicellulase, amylases and cellulases concentrated from culture media were added to flour in addition to other routine ingredients salt, sugar, yeast, additives etc., and the bread prepared was much softer and bigger in volume and size due to better leavening. The process increased the falling number (softening index) of the bread by more than 3–4 times. Various enzymes are used in baked goods and used for the specific purpose of softening, leavening for increasing palatability of the product and also for inhibiting staling, which is of considerable importance for increasing shelf life of bakery products.

13.2. Clarification of non-citrus fruit juice

An enzyme preparation from *Termitomyces clypeatus* containing a mixture of pectinase, cellulase, hemicellulase, arabinase and xylanase was useful for clarification of non-citrus fruit juice. The enzyme mixture was added to the apple juice at 40 °C and incubated for 2–4 h. The resulting suspension was filtered to a clear juice. A process for the preparation of an enzyme composition containing a mixture of pectinase and xylanase useful for clarification of non-citrus fruit juice was developed and patented (Sengupta, Sengupta, Naskar, & Jana, 1999). Pectinase is used in the extraction, clarification, filtration, and depectinization of fruit juices and wines by enzymatically breaking down the cell wall, for the maceration of fruits and vegetables, removal of the inner wall of lotus seed, garlic, almond, and peanut (Kashyap, Chandra, Kaul, & Tewari, 2000).

14. Concluding remarks and future prospects

Over the past few decades, there has been a strong upsurge of fungal community chiefly in the spheres of food, feed and therapeutics. Since most of these saprotrophic and mycorrhizal organisms can be genetically modified with ease, research initiatives like EUROFUNG currently underway have taken up strain improvement programmes with state of the art technologies to accomplish a unified objective of developing "secretion giants" out of these modest high potential organisms. In this regard, the optimization of production process deserves parallel importance. Although most current methods rely on submerged fermentation (SmF), the use of traditional solid state fermentation (SSF) processes should be explored more thoroughly. Several results have indicated that the SSF process results in improved levels of various secreted fungal hydrolases Pandey, Soccol, & Mitchell, 2000. Until now, most of the research in the field of SSF has been focused on process and fermentor design (Weber, Tramper, & Rinzema, 1999), treating the organism involved as a black box. However, with the advent of the "omics" age and ultra-modernization of farming methods and detection tools, it will actually be possible to compare the responses shown by these organisms towards changing habitat, growth parameters and nutritional status. Future use will not be confined solely to food production but will involve the employment of fungal community to cater for more and more diversified human needs. We have already seen successful ventures of this industry in nutraceuticals, food additives and condiments. This will expand even more with increased needs of treating human ailments, bioremediation and bio-fuel production. Currently, the mushroom industry has been structured in a view of providing considerable returns to the farmers. As such, both developed and developing countries have resorted to mushroom cultivation as one of the more promising options for increasing both rural income and earning of foreign currency. Consequently, the industry is under a process of decentralization to an accomodable extent. However, to maximize benefits for the respective countries, the farmers and industry owners need to be more altruistic. Cumulatively, this industry bears great potential to flourish as one the most expansive trade of all time.

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