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Food and Feed Enzymes

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Abstract Humans have benefited from the unique catalytic properties of enzymes, in particular for food production, for thousands of years. Prominent examples include the production of fermented alcoholic beverages, such as beer and wine, as well as bakery and dairy products. The chapter reviews the historic background of the development of modern enzyme technology and provides an overview of the industrial food and feed enzymes currently available on the world market. The chapter highlights enzyme applications for the improvement of resource efficiency, the biopreservation of food, and the treatment of food intolerances. Further topics address the improvement of food safety and food quality.

Keywords Biotechnology · Enzyme · Feed · Food · Phytase

Abbreviations

AMFEP	Association of Manufacturers and Formulators of Enzyme Products
BCWH	Bacterial cell wall hydrolase
BHA	2-*tert*-butyl-4-hydroxyanisole and 3-*tert*-butyl-4-hydroxyanisole
BHT	2,6-*bis*(1,1-dimethylethyl)-4-methylphenol
COT	British Committee on Toxicology
DCP	Dichloropropanol
EC	European Commission or Enzyme Commission
EFSA	European Food Safety Authority
GM	Genetically modified
JECFA	Joint FAO/WHO Expert Committee on Food Additives
MCPD	Monochloropropanediol
SCF	EU Scientific Committee on Food
StEP	Staggered extension process
TFA	Trans fatty acid

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Contents

1 A Brief History of Enzymes

Humans have used the unique catalytic properties of enzymes, in particular for food production, for thousands of years. One of the oldest surviving records of mankind, written approximately 4,500 years ago by the Sumerians and known as the *Epic of Gilgamesh* (Tablet II), refers to the positive aspects of the biotechnological products bread and beer:

Enkidu knew nothing about eating bread for food,
and of drinking beer he had not been taught.
The harlot spoke to Enkidu, saying:

"Eat the food, Enkidu, it is the way one lives.
Drink the beer, as is the custom of the land."

Enkidu ate the food until he was sated,
he drank the beer-seven jugs!—and became expansive and sang with joy!
He was elated and his face glowed.
He splashed his shaggy body with water,
and rubbed himself with oil, and turned into a human [1].

The oldest wine law can be found as a part of the Code of Hammurabi, eternalized on the famous stele of Hammurabi,[1] king of Babylon (1792–1750 BC). In addition, the ancient Greeks and Romans worshiped the gods Dionysus and Bacchus as being responsible for the success of fermentation processes. However, the actual reason for a reliable fermentation was not disclosed for a few hundred years.

The first direct observation and description of microorganisms was made by the Dutchman Antoni van Leeuwenhoek (1632–1723) in 1675 [2]. By using a simple

[1] The original stele is on exhibition in the Louvre in Paris.

homemade microscope, he was able to visualize microorganisms. Then 150 years later, in 1814, an important biotechnological reaction was described by Gottlieb Sigismund Kirchhoff (1764–1833). He noted that germinating grain contains a compound that converts starch into sugar [3]. Erhard Friedrich Leuchs (1800–1837) observed the same phenomenon for human saliva in 1831 [4].

Two years later, the French researchers Anselme Payen (1795–1871) and Jean-François Persoz (1805–1868) separated a substance from malt extract by means of alcohol precipitation that was capable of starch hydrolysis [5]. They called it diastase (amylase), derived from the Greek word *diastasis*, which means "separation". The French scientist Émile Duclaux (1840–1904) proposed to honor Payen and Persoz by introducing the suffix *-ase* as a naming convention for enzymes [6].

The Swedish chemist Jöns Jacob Berzelius (1779–1848) demonstrated that malt extract can break down starch more efficiently than sulphuric acid. Additionally, he coined the term "catalysis" [7]. The expression was derived from Greek words *kata,* which means "down", and *lyein,* which means "loosen".

Further contributing to our understanding of the fermentation process were the studies of Theodor Schwann (1810–1882), Friedrich Traugott Kützing (1807–1893), and especially of Louis Pasteur (1822–1895). Among other things, Schwann isolated animal pepsin to study it, and Pasteur provided the first experimental evidence for a microbial cause of fermentation [8]. In 1874, the Danish pharmacist Christian Ditlev Ammentorp Hansen (1843–1916) brought a standardized enzyme preparation (rennet) for the production of cheese on the market. The term *enzyme*—derived from Greek meaning "in yeast"—was suggested in 1876 by Wilhelm Friedrich Kühne (1837–1900) [9]. Friedrich Wilhelm Ostwald (1853–1932) discovered that enzymes are extremely important for chemical processes within living organisms and act as catalysts. In 1909, Ostwald was awarded the Nobel Prize in Chemistry "in recognition of his work on catalysis", among other contributions [10].

In 1894, the Japanese chemist Jōkichi Takamine (1854–1922) applied presumably for the first patent on a microbial enzyme in the United States [11]. A fungal enzyme was isolated from koji (*Aspergillus oryzae*) and was called takadiastase. Later, Takamine licensed the exclusive production rights for the enzyme to the Parke-Davis company. He became the "father of commercial enzymology"[2] and a millionaire. Takadiastase was sold as a digestive aid for the treatment of dyspepsia. Emil Fischer (1852–1919), a German chemist and Nobel Prize laureate of 1902 [12], published the Lock and Key Model for enzymes to visualize the substrate and enzyme interaction in 1894 [13].

Eduard Buchner's (1860–1917) investigations of the cell-free fermentation laid the cornerstone for modern enzymology [14]. He was able to prove that it was not the living yeast cells that were essential for fermentation, but rather certain

[2] Dr. Clifford W. Hesseltine (1991) said, "Dr. Jokichi Takamine was the father of commercial enzymology."

enzymes produced by the cells (no *vis vitalis* required). Buchner's results were in clear contradiction to the view of Pasteur, who presumed that the complex apparatus of intact yeast cells would be required. In 1907, Buchner was awarded the Nobel Prize in Chemistry "for his biochemical researches and his discovery of cell-free fermentation" [15].

The origin of modern enzyme kinetics dates back to 1903. Victor Henri (1872–1940) described the initial formation of an enzyme-substrate complex as an essential step in enzymatic reactions [16]. Inspired by his findings, Leonor Michaelis (1875–1949) and Maud Leonora Menten (1879–1960) developed the Michaelis–Menten equation 10 years later [17, 18]. In 1907, Otto Karl Julius Röhm (1876–1939) patented the first enzymes for large-scale industrial application: proteolytic enzymes extracted from cow stomachs, which brought significant technical advantages to the bating of hides for the production of leather. To produce and market the new product called Oropon, he established the company Röhm & Haas (Esslingen, Germany) together with his friend and businessman Otto Haas (1872–1960). In 1914, Otto Röhm applied for a patent for the first enzymatic detergent and named it Burnus. To distribute Burnus, Röhm & Haas acquired the soap factory August Jacobi and Son (Darmstadt, Germany) in 1916.

Sir Alexander Fleming (1881–1955), the discoverer of penicillin and Nobel Prize laureate of 1945 [19], coined the name *lysozyme* in 1922 for antibacterial enzymes isolated from hen egg white. James Batcheller Sumner (1887–1955) isolated the enzyme urease in 1926 and concluded that enzymes are proteins [20]. Twenty years later, he was awarded one-half of the Nobel Prize in Chemistry (1946) "for his discovery that enzymes can be crystallized" [21]. At the same time, Kaj Ulrik Linderstrøm-Lang (1896–1959) investigated chemical properties of proteins and laid down basic formalisms for their purification [22]. In 1929, Arthur Harden (1865–1940) and Hans von Euler-Chelpin (1873–1964) were awarded jointly the Nobel Prize in Chemistry "for their investigations on the fermentation of sugar and fermentative enzymes" [23]. Amongst others, they discovered the first coenzyme and called it "coferment".

Interestingly, it was the discovery of a class of enzymes, namely the DNA-cleaving enzymes (restriction endonucleases), that paved the way for modern molecular biology. For this pioneering work, the Swiss microbiologist and geneticist Werner Arber (born 1929) along with the American researchers Daniel Nathans (1928–1999) and Hamilton Othanel Smith (born 1931) were awarded the Nobel Prize in Physiology or Medicine in 1978 [24]. With the rapid development of modern molecular biotechnology, it was then possible to modify enzymes and to produce them recombinantly. The first commercial recombinant fat-splitting enzyme, a lipase from a genetically engineered fungal microorganism, was introduced in 1987 by the company Novo [25]. The fungal enzyme was called Lipolase and was directly used in the Japanese detergent Hi-Top made by the Lion Corporation.

The enormous technological advances in recent decades have led to the development of novel tools for molecular biotechnology and recombinant production systems. They gave new opportunities for the alteration of enzymatic

properties, which started almost 50 years ago by "chemical mutation" [26, 27]. At the end of the twentieth century, directed evolution by recombining DNA sequences of enzymes via DNA shuffling, error-prone polymerase chain reaction, or staggered extension process (StEP) resulted in enzymes with improved properties [28]. In addition to this more random mutational approach, the rational enzyme design was used for optimization of enzyme properties. On the basis of known amino acid sequences and crystallographic structures of enzymes, site-directed mutations have been made to delete or replace one or more amino acids to obtain improved catalytic properties [29].

Parallel to the molecular improvement of enzymes, new sources for novel and interesting enzymes were put into focus: the metagenomes [30]. Here, the genomic information of different habitats (e.g. soil, salt lakes, deep sea, and tree tops) is analyzed and transferred into host organisms for recombinant production of novel enzymes. Since then, the list of available enzymes for the production of food and animal feed has been growing constantly.

2 Legal Situation

Depending on the intended use of food enzymes, they can be divided into the following categories: food ingredients, food additives, and processing aids. Enzymes added for nutritional reasons are regarded as food ingredients, but this is rarely the case. Most enzymes are added to food for technological reasons. If these enzymes are still present in an active form in the end products, they fall under the definition of food additives. Otherwise, they commonly belong to the category of processing aids.

Prior to 2009 and partly still valid, the European regulations concerning enzymes were based mainly on four legislative acts: Directive 89/107/EEC, regulating food additives; Directive 83/417/EEC, regulating caseins and caseinates; Directive 2001/112/EC, regulating fruit juices and similar products; and Regulation (EC) 1493/1999, regulating the common organization of the wine market. Within the group of enzymes used for technological applications, only those enzymes considered to be food additives had to be assessed for safety before they were placed on the market. Local exceptions existed in Denmark and France, where national regulations for enzymes used as processing aids applied.

In 2008, the European Commission (EC) published a regulation that defined all food enzymes with technological purposes as a separate group and regulates them harmonized (Regulation (EC) No. 1332/2008). Together with regulations on food additives (Regulation (EC) No. 1333/2008), flavorings (Regulation (EC) No. 1334/2008), and a common authorization procedure (Regulation (EC) No. 1331/2008), these regulations are known as the so-called Food Improvement Agents Package. For the first time in the European history, the Food Improvement Agents Package provided a mandatory and harmonized authorization procedure for all food

enzymes with technological purposes.[3] A list of food enzymes was created and "only food enzymes included in the Community list may be placed on the market as such and used in foods […]".[4] By controlling the authorization procedure in terms of regulations instead of directives, the regulations are universally binding and directly applied. Modifications as part of the implementation process of directives by the Member States are not possible, which ensures legal certainty and should facilitate international trade.

Since the late 1980s, the use of genetic engineering in particular has revolutionized commercial enzyme production and massively accelerated the rate of innovation. New enzyme activities, new applications, and improved performance of existing enzymes were made possible. In this context, the increasing use of enzymes from extremophilic microorganisms played an important role, too. Additionally, genetic engineering permitted the production of enzymes from previously noncultivable microorganisms.

A possible downside of this new era of enzyme usage is the lack of long-term experience in matters of human consumption for many of these enzymes. Therefore, an important concern of the European legislature was to establish a universal procedure for the safety assessment of enzymes. In the past, risk assessments of food enzymes were carried out mainly by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) or were controlled by national authorization procedures. Very few reviews were also executed by the EU Scientific Committee on Food (SCF). Guidelines for the risk assessment of food enzymes have been published by the JECFA, the SCF, the Association of Manufacturers and Formulators of Enzyme Products (AMFEP) and the British Committee on Toxicology (COT).

This has changed after the entry of Regulation (EC) No. 1331/2008 into force. Only enzymes that were already authorized as food additives (E 1103 invertase and E 1105 lysozyme) or for the production of wine according to Regulation (EC) No. 1493/1999 (urease, β-glucanase, and lysozyme) will be automatically added to the positive list.[5] All other enzymes have to go through the newly established regulatory process. Therefore, the need for approval also includes those enzymes that were already approved under national laws (e.g. in France or in Denmark) or for which a positive evaluation of the JECFA exists. The approval process includes an assessment of health risks posed by the European Food Safety Authority (EFSA). The requirements for risk assessment are expressed as EFSA guidelines. To assess the need for technological benefit or misleading, however, no guidelines are provided.

The community list of enzymes shall include the name of the enzyme, its specifications (including origin, purity criteria, etc.), the foods to which the enzyme may be added, the conditions under which the enzyme may be used,

[3] Enzymes that are exclusively for the production of additives or the production of processing aids are, however, excluded from the new enzyme regulation.

[4] Regulation (EC) No. 1332/2008, Article 4.

[5] Regulation (EC) No. 1332/2008, Article 18(1).

selling restrictions, and specific requirements with respect to the labeling.[6] However, the positive list is not yet completed. Until then, the national provisions in force will continue to apply in the Member States.[7] It is assumed that the list will be completed by 2021. Additionally, certain transitional periods apply.[8]

3 Market Overview

3.1 Food Enzymes

For centuries, enzymes are used traditionally for dairy, baking, brewing, and winemaking, although not in isolated form. Enzymes are needed for cheese production and used for a wide variety of other dairy goods. Enzymes increase dough volume, lead to crispy crusts, and keep bread soft and fresh longer. They can be used to compensate for variations of flour and malt quality. In addition, breweries use enzymes to lower calories and alcohol concentration of beer. In winemaking, enzymes are used to maintain wine color and clarity or to reduce the sulfur content. Additionally, enzymes can enhance the filterability and improve the flavor of wine. They also help improving the quality, stability, and yield of fruit juices. Last but not least, the application of enzymes revolutionized the starch and sugar industry by making the hydrolysis of starch and rearrangement of glucose to fructose much more cost-effective.

The large number of applications makes enzymes highly valuable for the food and feed industry. According to the Novozymes Report 2011 [31], the global industrial enzyme market had a volume of approximately €2.7 billion. In the enzyme business,[9] food and beverage enzymes accounted for 29 % of sales, second only to household care enzymes (31 %). Feed and other technical enzymes accounted for 13 % of sales. With a market share of 47 % in industrial use enzymes, Novozymes was the global market leader, followed by DuPont (21 %), which recently acquired Danisco, and DSM (6 %). In the European market, AB Enzymes, Christian Hansen, and Henkel are prominent companies.

Of the six different existing enzyme classes (EC 1: oxidoreductases, EC 2: transferases, EC 3: hydrolases, EC 4: lyases, EC 5: isomerases, and EC 6: ligases) all but ligases are sold commercially for food and feed production. Currently, approximately 260 different enzymes are available in the European Union (Table 1). They can be isolated from plant[10] (3 %) and animal (6 %) materials but the majority are produced by means of fungi (filamentous ascomycetes and

[6] Regulation (EC) No. 1332/2008, Article 7.

[7] Regulation (EC) No. 1332/2008, Article 24.

[8] Regulation (EC) No. 1332/2008, Article 18(2).

[9] Including biobusiness.

[10] In relation to the total number of enzymes.

Table 1 List of enzymes used in the food and feed industry, together with their production strains and application areas [25, 32–36]

Accepted name	Systematic name	EC number	Production strains	Application areas
Oxidoreductases		*EC 1.x.x.x*		
Glucose oxidase	β-D-glucose:oxygen 1-oxidoreductase	EC 1.1.3.4	*Aspergillus niger, A. oryzae* (GM), *Penicillium chrysogenum*	Bakery, eggs, starch processing
Hexose oxidase	D-hexose:oxygen 1-oxidoreductase	EC 1.1.3.5	*Hansenula polymorpha* (GM)	Bakery, starch processing
Cellobiose dehydrogenase (acceptor)	Cellobiose:acceptor 1-oxidoreductase	EC 1.1.99.18	*Fusarium venenatum* (GM)	
Laccase	Benzenediol:oxygen oxidoreductase	EC 1.10.3.2	*Aspergillus niger, A. oryzae* (GM), *Trametes hirsuta, T. versicolor, Trichoderma longibrachiatum*	Beverages, cork treatment
L-ascorbate oxidase	L-ascorbate:oxygen oxidoreductase	EC 1.10.3.3	*Cucurbita pepo*	Clinical tests
Catalase	Hydrogen-peroxide:hydrogen-peroxide oxidoreductase	EC 1.11.1.6	*Aspergillus niger, A. oryzae* (GM), *Micrococcus luteus*	Beverages, eggs, starch processing, others
Peroxidase	Phenolic donor:hydrogen-peroxide oxidoreductase	EC 1.11.1.7	Lactoserum, soy	Starch processing, others
Linoleate 13*S*-lipoxygenase	Linoleate:oxygen 13-oxidoreductase	EC 1.13.11.12	soy, *Escherichia coli* (GM)	Bakery, flavor production
Transferases		*EC 2.x.x.x*		
Phosphatidylcholine-sterol *O*-acyltransferase	Phosphatidylcholine:sterol *O*-acyltransferase	EC 2.3.1.43	*Bacillus licheniformis* (GM)	Bakery, eggs, meat and fish, milk and cheese, others
Protein-glutamine γ-glutamyltransferase (transglutaminase)	Protein-glutamine γ-glutamyltransferase	EC 2.3.2.13	*Streptomyces mobaraensis*	Meat and fish, bakery, milk and cheese
Dextransucrase	Sucrose:(1→6)-α-D-glucan 6-α-D-glucosyltransferase	EC 2.4.1.5	*Leuconostoc mesenteroides*	Dextran production
1,4-α-glucan branching enzyme	(1→4)-α-D-glucan: (1→4)-α-D-glucan 6-α-D-[(1→4)-α-D-glucano]-transferase	EC 2.4.1.18	*Bacillus amyloliquefaciens* (GM)	Starch processing

(continued)

Table 1 (continued)

Accepted name	Systematic name	EC number	Production strains	Application areas
Cyclomaltodextrin glucanotrans-ferase	(1→4)-α-D-glucan 4-α-D-[(1→4)-α-D-glucano]-transferase (cyclizing)	EC 2.4.1.19	*Bacillus licheniformis* (GM)	Production of cyclodextrins
1,4-α-glucan 6-α-glucosyltrans-ferase	(1→4)-α-D-glucan:(1→4)-α-D-glucan(D-glucose) 6-α-D-glucosyltransferase	EC 2.4.1.24	*Aspergillus niger, Trichoderma longibrachiatum* (GM)	Starch processing
Hydrolases		*3.x.x.x*		
Carboxylesterase	Carboxylic-ester hydrolase	EC 3.1.1.1	*Rhizomucor miehei*	Hydrolysis of various carboxylic esters
Triacylglycerol lipase	Triacylglycerol acylhydrolase	EC 3.1.1.3	*Aspergillus niger* (GM), *A. oryzae* (GM), *Bacillus licheniformis* (GM), calf, *Candida lipolytica, C. rugosa,* goat, *Hansenula polymorpha* (GM), lamb, *Mucor javanicus, Penicillium roqueforti, Pichia angusta* (GM), *Rhizomucor miehei, Rhizopus niveus* (non-GM & GM), *R. oryzae*	Bakery, milk and cheese, starch processing, others
Phospholipase A_2	Phosphatidylcholine 2-acylhydrolase	EC 3.1.1.4	*Aspergillus oryzae* (GM), *A. niger* (GM), ox, pig, *Streptomyces violaceoruber* (non-GM and GM), *Trichoderma longibrachiatum* (GM)	Bakery, eggs, starch processing, others
Lysophospholipase	2-lysophosphatidyl-choline acylhydrolase	EC 3.1.1.5	*Aspergillus niger* (non-GM and GM), *Trichoderma longibrachiatum* (GM)	Beverages, starch processing
Pectinesterase	Pectin pectylhydrolase	EC 3.1.1.11	*Aspergillus niger* (non-GM and GM), *A. oryzae* (GM), *A. sojae, Penicillium funiculosum, Rhizopus oryzae, Trichoderma longibrachiatum* (GM)	Beverages, fruits and vegetables
Tannase	Tannin acylhydrolase	EC 3.1.1.20	*Aspergillus oryzae, A. niger*	Beverages

(continued)

Table 1 (continued)

Accepted name	Systematic name	EC number	Production strains	Application areas
Acylglycerol lipase	Glycerol-ester acylhydrolase	EC 3.1.1.23	*Penicillium camembertii*	Lipids
Phospholipase A_1	Phosphatidylcholine 1-acylhydrolase	EC 3.1.1.32	*Aspergillus oryzae* (GM)	Milk and cheese
Feruloyl esterase	4-hydroxy-3-methoxycinnamoyl-sugar hydrolase	EC 3.1.1.73	*Aspergillus niger, Streptomyces werraensis*	Biomass degradation
Hydroxyacylglutathione hydrolase	*S*-(2-hydroxyacyl)-glutathione hydrolase	EC 3.1.2.6	*Bacillus amyloliquefaciens* (GM)	Bakery
Phytase (3-, 4-)	*Myo*-inositol-hexakisphosphate x-phosphohydrolase	EC 3.1.3.8/26	*Aspergillus niger* (non-GM and GM), *A. oryzae (GM), Schizosaccharomyces pombe* (GM), *Trichoderma longibrachiatum* (GM)	Feed, bakery
Phosphodiesterase I	Oligonucleotide 5′-nucleotidohydrolase	EC 3.1.4.1	*Leptographium procerum,* malt, *Penicillium citrinum*	Yeast extract production
Phospholipase C	Phosphatidylcholine cholinephosphohydrolase	EC 3.1.4.3	*Pichia pastoris* (GM)	Emulsifiers
Ribonuclease P		EC 3.1.26.5	*Penicillium citrinum*	Yeast extract production
α-Amylase	4-α-D-glucan glucanohydrolase	EC 3.2.1.1	*Aspergillus niger, A. oryzae, Bacillus amyloliquefaciens* (non-GM and GM), *B. licheniformis* (non-GM and GM), *B. stearothermophilus, Microbacterium imperiale, Pseudomonas fluorescens* (GM), *Trichoderma longibrachiatum* (GM)	Beverages, bakery, starch processing
β-Amylase	4-α-D-glucan maltohydrolase	EC 3.2.1.2	Barley, *Penicillium multicolor*, wheat, soy	Beverages, starch processing

(continued)

Table 1 (continued)

Accepted name	Systematic name	EC number	Production strains	Application areas
Glucan 1,4-α-glucosidase	4-α-D-glucan glucohydrolase	EC 3.2.1.3	*Aspergillus niger* (non-GM and GM), *A. oryzae, Hypocrea jecorina* (GM), *Rhizopus niveus R. oryzae, Trichoderma longibrachiatum* (GM)	Bakery, beverages, starch processing
Cellulase	4-(1,3;1,4)-β-D-glucan 4-glucanohydrolase	EC 3.2.1.4	*Aspergillus niger, Humicola insolens, Hypocrea jecorina* (GM), *Penicillium funiculosum, Talaromyces emersonii, Trichoderma longibrachiatum* (non-GM and GM), *T. viride*	Bakery, beverages, starch processing
Endo-1,3(4)-β-glucanase	3-(1→3;1→4)-β-D-glucan 3(4)-glucanohydrolase	EC 3.2.1.6	*Aspergillus niger, A. oryzae* (GM), *Bacillus amyloliquefaciens* (non-GM and GM), *Cellulosimicrobium cellulans, Disporotrichum dimorphosporum, Humicola insolens, Penicillium funiculosum, Talaromyces emersonii, Trichoderma longibrachiatum* (non-GM and GM)	Beverages
Inulinase	1-β-D-fructan fructanohydrolase	EC 3.2.1.7	*Aspergillus niger, A. oryzae* (GM)	Starch processing
Endo-1,4-β-xylanase	4-β-D-xylan xylanohydrolase	EC 3.2.1.8	*Aspergillus niger* (non-GM and GM), *A. oryzae* (GM), *Bacillus amyloliquefaciens* (GM), *B. licheniformis* (GM), *Disporotrichum dimorphosporum, Humicola insolens, Penicillium funiculosum, Talaromyces emersonii, Trichoderma longibrachiatum* (non-GM and GM), *T. viride*	Bakery, beverages, starch processing, others

(continued)

Table 1 (continued)

Accepted name	Systematic name	EC number	Production strains	Application areas
Dextranase	6-α-D-glucan 6-glucanohydrolase	EC 3.2.1.11	*Chaetomium erraticum, C. gracile, Penicillum lilacinum*	Sugar refinery
Chitinase	(1→4)-2-acetamido-2-deoxy-β-D-glucan glycanohydrolase	EC 3.2.1.14	*Streptomyces violaceoruber* (GM)	Production of *N*-acetyl-glucosamine
Polygalacturonase	(1→4)-α-D-galacturonan glycanohydrolase	EC 3.2.1.15	*Aspergillus niger* (non-GM and GM), *A. wentii, Rhizopus oryzae, Trichoderma longibrachiatum*	Beverages
Lysozyme	Peptidoglycan *N*-acetylmuramoylhydrolase	EC 3.2.1.17	Chicken egg	Preservation
α-Glucosidase	α-D-glucoside glucohydrolase	EC 3.2.1.20	*Aspergillus niger*	Starch processing
β-Glucosidase	β-D-glucoside glucohydrolase	EC 3.2.1.21	*Aspergillus niger*	Sugar specialties
α-Galactosidase	α-D-galactoside galactohydrolase	EC 3.2.1.22	*Aspegillus niger, A. oryzae* (GM), *Saccharomyces cerevisiae* (GM)	Medical applications
β-Galactosidase (lactase)	β-D-galactoside galactohydrolase	EC 3.2.1.23	*Aspergillus niger, A. oryzae* (non-GM and GM), *Bacillus circulans, Kluyveromyces fragilis, K. lactis* (non-GM and GM)	Milk processing
β-Fructofuranosidase (invertase)	β-D-fructofuranoside fructohydrolase	EC 3.2.1.26	*Aspergillus niger, Saccharomyces cerevisiae*	Sucrose processing
Pullulanase	Pullulan 6-α-glucanohydrolase	EC 3.2.1.41	*Bacillus acido-pullulyticus, B. amyloliquefaciens* (GM), *B. brevis, B. licheniformis* (GM), *B. subtilis* (GM), *Klebsiella planticola, Pullulanibacillus sp., Trichoderma longibrachiatum* (GM)	Beverages, starch processing
β-L-*N*-acetylhexos-amini-dase	β-*N*-acetyl-D-hexosaminide *N*-acetylhexosaminohydrolase	EC 3.2.1.52	*Streptomyces violaceoruber* (GM)	

(continued)

Table 1 (continued)

Accepted name	Systematic name	EC number	Production strains	Application areas
α-*N*-arabinofuranosidase	α-L-arabinofuranoside arabinofuranohydrolase	EC 3.2.1.55	*Aspergillus niger* (non-GM and GM)	Bakery
Glucan 1,3-β-glucosidase	3-β-D-glucan glucohydrolase	EC 3.2.1.58	*Penicillium funiculosum, Trichoderma harzianum*	Starch processing
Glucan 1,4-α-maltotetrao-hydrolase	4-α-D-glucan maltotetraohydrolase	EC 3.2.1.60	*Bacillus licheniformis* (GM), *B. subtilis* (GM)	Flour processing
Mycodextranase	(1→3)-(1→4)-α-D-glucan 4-glucanohydrolase	EC 3.2.1.61	*Bacillus licheniformis* (GM)	Bakery
Isoamylase	Glycogen α-1,6-glucano-hydrolase	EC 3.2.1.68	*Pseudomonas amyloderamosa*	Starch processing
Mannan *endo*-1,4-β-mannosidase	1,4-β-D-mannan mannanohydrolase	EC 3.2.1.78	*Aspergillus niger, Bacillus halodurans, Trichoderma longibrachiatum* (GM)	Processing of mannans and galacto-mannans
Arabinan *endo*-1,5-α-L-arabinanase	5-α-L-arabinan 5-α-L-arabinanohydrolase	EC 3.2.1.99	*Aspergillus niger*	Processing of L-arabinan
Glucan 1,4-α-malto-hydrolase	4-α-D-glucan α-maltohydrolase	EC 3.2.1.133	*Bacillus amyloliquefaciens* (GM), *Microbacterium imperiale*	Bakery, starch processing
Aminopeptidase		EC 3.4.11.x	*Aspergillus niger, A. oryzae, Rhizopus oryzae*	Milk processing
Leucyl aminopeptidase		EC 3.4.11.1	*Aspergillus oryzae*	Beverages, production of soy sauce
Serine-type carboxypeptidase		EC 3.4.16.x	*Aspergillus niger* (GM)	Meat and fish, milk processing, flavoring preparations
Serine endopeptidase		EC 3.4.21.x	*Aspergillus oryzae, A. wentii, Bacillus amyloliquefaciens, B. licheniformis, Cryphonectria parasitica, Fusarium venenatum* (GM), *Rhizomucor miehei*	Protein hydrolysis
Chymotrypsin		EC 3.4.21.1	*Bacillus licheniformis* (GM), beef pancreas	Protein hydrolysis

(continued)

Table 1 (continued)

Accepted name	Systematic name	EC number	Production strains	Application areas
Trypsin		EC 3.4.21.4	*Fusarium venenatum* (GM)	Milk and cheese
Thrombin		EC 3.4.21.5	Cattle, pig	Meat and fish
Prolyl oligopeptidase		EC 3.4.21.26	*Aspergillus niger* (GM)	Beverages, others
Subtilisin		EC 3.4.21.62	*Bacillus licheniformis*	Protein hydrolysis
Oryzin		EC 3.4.21.63	*Aspergillus oryzae*	Bakery
Aqualysin 1		EC 3.4.21.111	*Bacillus amyloliquefaciens* (GM)	Bakery
Papain		EC 3.4.22.2	*Carica papaya*	Beverages, others
Aspartic endopeptidases		EC 3.4.23.x	Animal origin, *Aspergillus niger* (GM), *A. oryzae, A. wentii, Bacillus licheniformis, Micrococcus caseolyticus*	Bakery, beverages, milk and cheese, protein hydrolysis
Pepsin		EC 3.4.23.1/2	Cattle, pig	Protein hydrolysis
Pepsin A		EC 3.4.23.1	Bovine rennet	Milk and cheese
Chymosin		EC 3.4.23.4	*Aspergillus niger* (GM), calf, *Escherichia coli* (GM), *Kluyveromyces lactis* (GM)	Milk and cheese, others
Aspergillopepsin I		EC 3.4.23.18	*Aspergillus oryzae* (non-GM and GM), *A. wentii*	Milk and cheese, others
Endothiapepsin		EC 3.4.23.22	*Cryphonectria parasitica*	Others
Mucorpepsin		EC 3.4.23.23	*Aspergillus oryzae* (GM), *Mucor pusillus, Rhizomucor miehei*	Meat and fish, protein hydrolysis
Thermolysin		EC 3.4.24.27	*Geobacillus caldoproteolyticus*	Protein hydrolysis
Bacillolysin		EC 3.4.24.28	*Bacillus amyloliquefaciens* (non-GM and GM)	Bakery, beverages, meat and fish, flour processing, protein hydrolysis
Deuterolysin		EC 3.4.24.39	*Aspergillus oryzae, A. wentii*	Beverages, protein hydrolysis

(continued)

Table 1 (continued)

Accepted name	Systematic name	EC number	Production strains	Application areas
Asparaginase	L-asparagine amidohydrolase	EC 3.5.1.1	*Aspergillus niger* (GM), *A. oryzae* (GM)	Potato products, bakery, fruits and vegetables, starch processing, others
Glutaminase	L-glutamine amidohydrolase	EC 3.5.1.2	*Aspergillus niger, Bacillus amyloliquefaciens, B. subtilis*	Protein hydrolysis
Urease	Urea amidohydrolase	EC 3.5.1.5	*Lactobaillus fermentum*	Others
Protein-glutamine glutaminase	Protein-L-glutamine amidohydrolase	EC 3.5.1.44	*Chryseobacterium proteolyticum*	Production of seasonings
AMP deaminase	AMP aminohydrolase	EC 3.5.4.6	*Aspergillus melleus, A. oryzae*	Yeast extract
Lyases		*EC 4.x.x.x*		
Pectate lyase	(1→4)-α-D-galacturonan lyase	EC 4.2.2.2	*Bacillus subtilis*	Beverages
Poly(β-D-mannuronate) lyase	Poly[(1→4)-β-D-mannuronide] lyase	EC 4.2.2.3	*Sphingobacterium multivorum*	Processing of alginates
Acetolactate decarboxylase	(2*S*)-2-hydroxy-2-methyl-3-oxobutanoate carboxy-lyase [(3*R*)-3-hydroxybutan-2-one-forming]	EC 4.1.1.5	*Bacillus amyloliquefaciens* (GM), *Saccharomyces cerevisiae* (GM)	Beverages
Pectin lyase	(1→4)-6-*O*-methyl-α-D-galacturonan lyase	EC 4.2.2.10	*Aspergillus niger* (non-GM and GM), *A. sojae, Penicillium funiculosum, Rhizopus oryzae, Trichoderma longibrachiatum* (GM)	Beverages
Isomerases		*EC 5.x.x.x*		
Glucose isomerase		EC 5.3.1.x	*Actinoplanes missouriensis, Bacillus coagulans, Streptomyces murinus, S. olivochromogenes*	Sugar production
Xylose isomerase	D-xylose aldose-ketose-isomerase	EC 5.3.1.5	*Streptomyces murinus, S. olivochromogenes, S. rubiginosus* (non-GM and GM)	Sugar production
Glucose-6-phosphate isomerase	D-glucose-6-phosphate aldose-ketose-isomerase	EC 5.3.1.9	*Streptomyces violaceoniger*	Sugar production

GM genetically modified

basidiomycetes: 58 %, yeasts: 5 %) and bacteria (28 %). One-third of the offered enzymes originate from genetically modified organisms.[11]

3.2 Feed Enzymes

In addition to a variety of applications in the food industry, enzymes may also be used during the manufacture of feed. In this capacity, they are used primarily to increase the availability of essential nutrients, complementing the spectra of activities of enzymes already present in feed and animals' own digestive enzymes. Thus, the animal feedstuff can be used more efficiently and the use of enzymes helps to conserve resources and avoid waste. The most important enzyme in the feed sector is phytase (see 4.1.3). In addition, xylanase and β-glucanase are of importance.

4 Applications

The manifold industrial applications of enzymes have been reviewed in great detail [e.g. 37, 38]. Very good overviews on enzymes used in food and feed technology were published by Whitehurst and van Oort [39] and Bedford and Partridge [40]. Current trends have been reviewed by Son and Ravindran [41].

Due to the existing literature and the enormous variety of enzymes used in food and animal feed (Table 1), it is not necessary or even possible to cover all applications in the context of this book chapter. Therefore, a number of prominent examples in the areas of resource efficiency, biopreservation, health, safety, and food quality are highlighted here.

4.1 Resource Efficiency

4.1.1 Amylase

Due to the increasing availability of efficient and stable biocatalysts, chemical processes are more and more replaced by biotechnological methods. The latter often show superior economic and ecological operating figures. Therefore, the acid-catalyzed saccharification and the production of sugar specialties have been replaced worldwide by enzymatic procedures. In these systems, amylases are

[11] Counting self-cloned organisms, although legal self-cloning of nonpathogenic naturally occurring microorganisms is excluded from Directive 90/219/EEC.

essential and thus have become one of the most important industrial enzymes [42]. Besides the conversion of starch to sugar syrups, they can be used in other areas of the food industry, as well as in the detergent, textile, paper, and pharmaceutical industries [37, 43, 44].

α-Amylases (4-α-D-glucan glucanohydrolase, EC 3.2.1.1) degrade starch and similar carbohydrates by endohydrolysis of their (1→4)-α-D-glucosidic bonds. The majority of α-amylases belong to the group of metalloenzymes and require calcium ions (Ca^{2+}). By means of direct evolution, the performance of several amylases was further maximized and adapted to the needs of the starch processing industry [45]. The enhancement of the thermostability of amylase was achieved by DNA-shuffling techniques [46]. The baking industry and consumers might benefit from genetically optimized starch-modifying enzymes. Based on the improved thermal stability of an α-amylase in the acidic pH range, the retrogradation of sourdough breads ("staling") can be delayed [47].

4.1.2 Peptidase

Seasonings are biotechnologically obtained by hydrolysis of vegetable proteins in multistage enzymatic processes. Unlike the previously common catalysis with the aid of hydrochloric acid, which leads to the presence of small remainders of fat in the formation of monochloropropanediols (mainly 3-MCPD) and dichloropropanols (1,3- and 1,2-DCP) [48], no toxic byproducts are produced in the enzymatic process. Peptidases (EC 3.4) from edible mushrooms with new catalytic properties allow for an even more efficient protein hydrolysis [49].

4.1.3 Phytase

Phytases are phosphatases able to hydrolyze O–P bonds in phytic acid liberating inorganic phosphate. Phytases can be grouped according to the attack on the hexaphosphoric ester into 3-phytase (*myo*-inositol-hexakisphosphate 3-phosphohydrolase, EC 3.1.3.8) as well as 4-phytase (*myo*-inositol-hexakisphosphate 4-phosphohydrolase, EC 3.1.3.26), releasing the phosphate at the corresponding position at the inositol ring (Fig. 1).

Phytic acid is used by plants to store different type of anions (Cu^{2+}, $Fe^{2+/3+}$, Ca^{2+}, Mg^{2+}, etc.). The resulting salts are known as phytates. Nonruminant animals do not have the enzymatic ability to hydrolyze phytates; therefore, phosphate and minerals are not absorbed, but rather they pass through the intestinal tract undigested. The addition of phytases to food and feed does therefore enhance the availability of phosphate and minerals bound in phytates.

More than 20 years ago, the first commercial phytase product for feed, Natuphos, was released [50]. Nowadays, several other phytase products are available on the market for the improvement of animal feed, such as Ronozyme from Novozymes and Finase from AB Enzymes. The range of applications in nonruminant

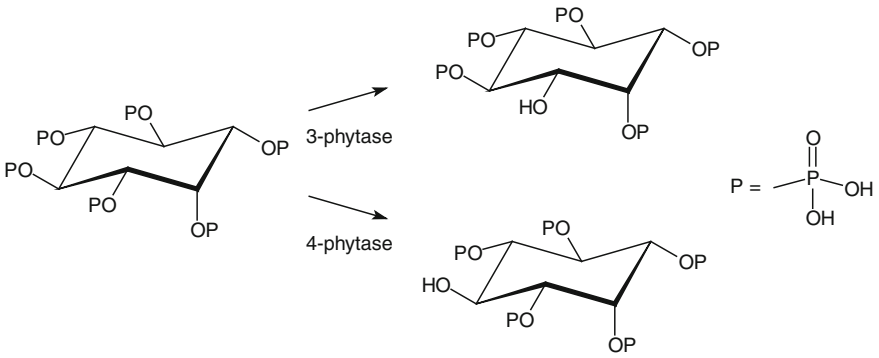

Fig. 1 Hydrolysis of *myo*-inositol-1,2,3,4,5,6-hexakisphosphate by 3- and 4-phytase

feed is large, as discussed in several reviews on the usage of phytases in the fodder of pigs [51, 52], poultry [53], and fish [54, 55]. Additionally, phytase might also be used in food for the improvement of the nutritional value of cereal food products by degrading phytate [56].

Commercial recombinant production of phytases occurs generally, if not exclusively, in ascomycetes [32, 50], whereas the phytase genes originate from different phyla: bacteria, ascomycetes, and basidiomycetes (*Peniophora lycii* [57]). Industrial production takes place in liquid media in stirred bioreactors on a $6 \times 120\ m^3$ scale. In addition, the cultivation of phytase producing filamentous fungi in solid-state [58] or solid-substrate [59] fermentation systems has also been studied, but it lacks industrial adaptability. Another opportunity for the production of phytases is the usage of transgenic plants, such as maize, rice, soybean, and wheat [60–63].

4.2 Biopreservation

Foods that spoil during manufacturing or storage endanger human life, waste important resources, and cost the food industry vast sums of money. Therefore, foods have been preserved since the dawn of mankind. In recent decades, this has often been achieved by adding antimicrobial preservatives (e.g. sulfites, sorbic acid, benzoic acid, and their salts) and/or antioxidants (e.g. 2-*tert*-butyl-4-hydroxyanisole and 3-*tert*-butyl-4-hydroxyanisole (BHA), 2,6-*bis*(1,1-dimethyl-ethyl)-4-methylphenol (BHT)). In particular, synthetic food additives are often disliked by consumers because they are occasionally considered critically [64].

Alternatively, natural or controlled microbiota or antimicrobials can be used to enhance the safety and to extend the shelf life of food [65]. Lactic acid bacteria are commonly used. They often produce lactic and acetic acid, hydrogen peroxide, and under certain conditions peptide bacteriocins, which prevent the development of

pathogens and spoilage microorganisms [66, 67]. The most important bacteriocin is the peptide nisin [68], as discussed in detail in Chap. 2

4.2.1 Lysozyme

The cell wall of bacteria is composed of the peptidoglycan murein. Murein is a cross-linked heteropolymer consisting of sugars and amino acids. Its task is to maintain the turgor pressure and shape of the bacterial cell. Peptidoglycan can be cleaved by bacterial cell wall hydrolases (BCWHs), which leads to bacteriolysis. BCWHs are found ubiquitously in nature in animals, plants, protozoa, bacteria, and bacterial viruses [69]. Due to their high specificity, they only attack bacteria.

The most important and best characterized BCWH is lysozyme (peptidoglycan *N*-acetylmuramoylhydrolase, EC 3.2.1.17) from hen egg white. Lysozyme was discovered by Sir Alexander Fleming in 1922 and has been extensively studied since then. It became one of the few approved natural antimicrobials for use in food in the European Union (E 1105). The main application of lysozyme is to prevent the growth of gram-positive bacteria in semi-hard cheeses (e.g. to prevent late blowing caused by *Clostridium tyrobutyricum*) [70]. Further applications include spoilage control in wine [71], beer [72], fish [73], and meat [74].

4.3 Health, Safety and Quality

4.3.1 Lactase

β-D-Galactosidase (*β*-D-galactoside galactohydrolase, EC 3.2.1.23) hydrolyses the glycosidic bond of the disaccharide lactose into its monomers glucose and galactose. Therefore, *β*-D-galactosidase is also known as lactase. *β*-D-Galactosidases can be found throughout nature. Commercial enzymes are mainly of fungal origin of the genera *Kluyveromyces* (yeast) and *Aspergillus* (filamentous ascomycete) [75]. Although several *β*-D-galactosidases are available on the market, new efforts are still conducted to improve the enzymatic properties by site-directed mutagenesis [76] or to find new *β*-D-galactosidase genes by screening metagenome databases [77].

From a technological point of view, the milk sugar lactose is hydrolyzed because of the resulting increase of sweetness and the reduced susceptibility to crystallization during spray drying of milk and whey [78, 79]. Nevertheless, the most apparent application is the production of “lactose-free” milk products, which enables the consumption of dairy products by lactose-intolerant people.

For industrial processes, *β*-D-galactosidases can be applied by immobilization of the enzyme on carriers, such as cellulose, alginate, or other polymers, for hydrolysis of milk or whey products [80]. Conversion rates of lactose in batch and continuous operation mode by immobilized *β*-D-galactosidase might reach 95 %

[81]. To reach even lower lactose concentrations of less than 0.01 %, a combination of β-D-galactosidase activity and ultrafiltration as well as nanofiltration methods can be used [82]. Besides the degradation of substances that lead to food intolerances, the elimination of food allergens by means of a specific degradation of allergenic epitopes is another promising application of enzymes [83].

4.3.2 Asparaginase

Using the enzyme asparaginase (L-asparagine amidohydrolase, EC 3.5.1.1), it is possible to significantly reduce the formation of the cooking carcinogen acrylamide during roasting, deep-frying, or baking of foods [84]. The enzyme hydrolyzes free asparagine to aspartic acid, thereby preventing the formation of acrylamide by reaction of asparagine with reducing sugars at elevated temperatures during the Maillard reaction (Fig. 2) [85].

The mitigation of acrylamide formation is especially important for a number of cereal- and potato-based products, including crackers, crispbread, gingerbread, biscuits, French fries, and potato chips. After asparaginase pretreatment, the acrylamide concentration of certain foods could be reduced by up to 97 % [84, 86]. By means of in vitro directed evolution, the properties of asparaginase were optimized. For example, an Asp133Leu mutation of a wild-type enzyme showed a significantly improved thermal stability. The enzyme's half-life at 50 °C increased from 3 to 160 h, and the half-inactivation temperature of the mutant was 9 °C higher.

4.3.3 Lipase

Trans fatty acids (TFAs) are fatty acids with at least one double bond in *(E)*-configuration. The consumption of TFAs increases the risk of coronary heart diseases. Thus, their concentrations in lipid-containing products should be reduced [87]. Naturally, TFAs occur in small amounts in meat and milk of ruminants, but the most significant concentrations of TFAs develop during partial hydrogenation and deodorization of fats [88]. The formation of TFAs during fat hardening can be avoided by lipase catalyzed transesterification to increase the slip melting points of fats. [89].

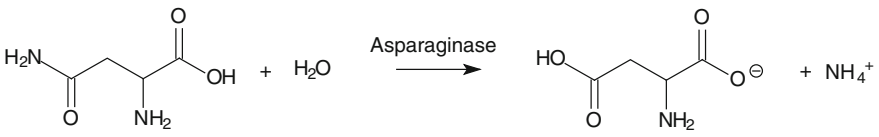

Fig. 2 Asparaginase catalyzed hydrolysis of asparagine to aspartic acid

For enzymatic transesterification between different lipids, triacylglycerol lipases (triacylglycerol acylhydrolase, EC 3.1.1.3) acting on the SN1 and SN3 positions of the triglyceride are used. Various lipases have been applied for the production of table margarine out of fat-oil blends. Lipases of the ascomycetes *Thermomyces lanuginosa* and *Rhizomucor miehei*, as well as a lipase of the proteobacterium *Pseudomonas* sp., were used for transesterification of fat blends consisting of palm stearin and vegetable oil [90, 91]. Fully hydrogenated oils in blends with vegetable oils also have been used [92]. In all studies, an increase of the slip melting points and the solid fat content was achieved in the fat-oil blend, thus indicating an alternative method for fat hardening via hydrogenation.

4.4 Further Applications

4.4.1 Laccase

Laccases are multicopper-oxidases (benzenediol:oxygen oxidoreductase, EC 1.10.3.2) that are able to oxidize phenolic substrates (e.g. 2,6-dimethoxy-phenol), aromatic amines (e.g. 1-hydroxybenzotriazole), or polycyclic aromatic hydrocarbons (e.g. anthracene) [93]. The oxidation of the substrate occurs via a one-electron reduction and is accompanied by a reduction of molecular oxygen to water.

Most laccases are of fungal origin [94], but they also occur in bacteria, insects, and plants [95–97]. Due to the broad substrate range of laccases, their possible industrial usage is widespread. Nevertheless, only few applications have been commercialized up to now, mostly in the textile industry [98]. In the food and feed sector, laccases have been evaluated for different applications, such as the stabilization of beverages, the reduction of off-flavors, the improvement of wheat dough, and the usage of laccases as biosensors in the food processing industry [37, 98–100].

Off-flavors in wine may occur due to microbial conversion of phenolic compounds present in the wine itself or in the cork stoppers. In a commercial product from Novozymes called Suberase, laccase is used for polymerization of phenolic compounds in the cork, which act as precursors for malodors, such as pentachlorophenol or 2,4,6-trichloroanisol [101].

In apple juice, addition of laccase reduced the amount of the phenolic off-flavors 2,6-dibromophenol, guaiacol, and α-terpineol [102]. Several studies used laccases to increase the stability of apple juices by polymerization of phenols and their subsequent removal by ultrafiltration methods [103]. A positive side effect is the decrease of molecular oxygen in the juice due to its consumption by laccases. Nevertheless, desired phenols might also be oxidized; thus, the sensory attributes and nutritional value might be altered.

The polymerization reactions catalyzed by laccases can also be used to improve the shade of food, such as the coloration of tea-based products [104]. In the bakery

industry, laccase might be used together with proteases or xylanases to improve the dough quality. It was proposed that laccases oxidize ferulic acid attached to the arabinoxylan present in cereal flour. The obtained phenolic radicals can undergo a nonenzymatic reaction, resulting in cross-linked feruloylated arabinoxylans [105]. In oat flour-based bread, the usage of laccase increased the loaf-specific volume and decreased the crumb hardness [106]. Contradictory, in another study, laccase alone decreased the specific volume and increased the crumb hardness [105]. A combined usage of laccase with xylanase improved again the oat flour bread properties [105, 107].

4.4.2 Peroxidase

Peroxidases (EC 1.11.1.x) are a diverse group of oxidoreductases using peroxide as an electron acceptor. Their substrate spectrum ranges from hydrogen peroxide decomposed by catalase (hydrogen-peroxide:hydrogen-peroxide oxidoreductase, EC 1.11.1.6), phenolic compounds degraded by Mn-dependent and lignin peroxidases (Mn(II):hydrogen-peroxide oxidoreductase, EC 1.11.1.13 and 1,2-*bis* (3,4-dimethoxyphenyl)propane-1,3-diol:hydrogen-peroxide oxidoreductase, EC 1.11.1.14), and recalcitrant dyes by DyP-type peroxidases (reactive-blue-5:hydrogen-peroxide oxidoreductase, EC 1.11.1.19).

Direct supplementation of peroxidases to food was applied for the degradation of carotenoids used for coloring of cheese. The whey resulting from colored cheese production has an orange-yellowish tint, which interferes with further usage of the whey. Recently, a fungal peroxidase of the DyP-type was commercialized for bleaching of this kind of whey fluid under the name MaxiBright (DSM). To generate the hydrogen peroxide required by the peroxidase as a cofactor in situ, a glucose oxidase and a β-galactosidase were employed as auxiliary enzymes [108] (Fig. 3).

4.4.3 Lipoxygenase

Microorganisms, fungi, plants, and their enzymes can be used to synthesize natural flavor compounds [109]. Probably the most important example is the biotechnological production of the highly sought after vanillin [110]. Recently, disrupted

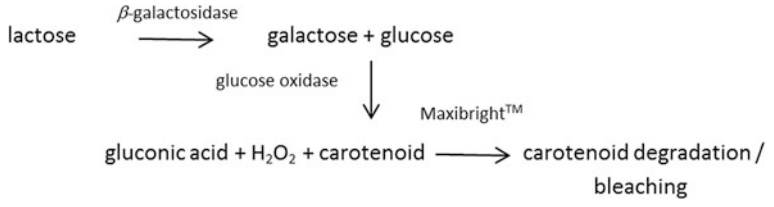

Fig. 3 Bleaching of whey and milk by a multiple enzyme system (modified from [108])

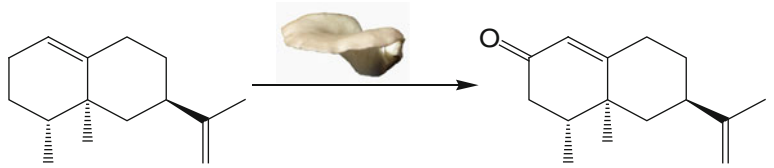

Fig. 4 Oxidation of (+)-valence to the grapefruit flavor (+)-nootkatone by lyophilisates of the basidiomycete *Pleurotus sapidus*

cells of the edible basidiomycete *Pleurotus sapidus* were deployed as a potent biocatalyst for the transformation of (+)-valencene to natural (+)-nootkatone [111] (Fig. 4).

The enzyme responsible for the biotransformation was biochemically characterized and purified, and the enzyme encoding cDNA was amplified from a cDNA library by polymerase chain reaction [112]. The catalytic reaction sequence of the enzyme was further investigated and a lipoxygenase-type oxidation of (+)-valencene via secondary and tertiary hydroperoxides was suggested [113]. In ongoing research, the dioxygenase was heterologously expressed in the cytosol and periplasm of *Escherichia coli* [114]. Only recently, the enzyme was identified as a potent 13*S*-lipoxygenase ($LOX_{Psa}1$; linoleate:oxygen 13-oxidoreductase, EC 1.13.11.12), and the kinetic parameters of the recombinant enzyme were determined by using linoleic acid as the substrate [115].

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