22 Enzymes and Flavour Biotechnology

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22.1 Introduction

There are about 25,000 enzymes present in nature and about 400 have been commercialised mainly for stereoselective organic synthesis and also for the biotechnological production of flavour compounds. The worldwide market for enzymes is more than US $1 billion.

The majority of enzymes in food biotechnology comprise hydrolytic enzymes, transferases, oxireductases and lyases.

Microbial enzymes play the greatest role in production of flavour compounds; they can also be expressed in recombinant microorganisms.

This chapter is not only an update of our review of 1997 [1] but also an overview of the latest development in enzyme-based flavour technology. Some aspects of the present chapter are based on the previous review [1].

22.2 Hydrolytic Enzymes

22.2.1 Lipases (EC 3.1.1.X)

Lipases are serine hydrolases that catalyse the hydrolysis of lipids to fatty acids and glycerol [2]. In contrast to esterases, they work at the lipid–water interface and show only little activity in aqueous solutions. Studies of the X-ray structures of human lipase [3,4] and *Mucor miehei* lipase [5,6] revealed a change in conformation at the lipid–water interface, which explains the increase of activity.

Both of the aforementioned lipases contain Asp-His-Ser triades; different catalytic triades can be found, e.g., in *Geotrichum candidum* (Glu-His-Ser) [7] or in *Humicola lanuginosa* (Asp-His-Tyr) [8].

Lipases play an important role in organic synthesis and also in flavour biotechnology. Pig pancreatic extract and especially many microbial lipases are used for ester hydrolysis, esterification (alcohol and acid), transesterification (ester and

alcohol), interesterification (ester and acid) and transfer of acyl groups from esters to other nucleophiles like amines or thiols [1].

Some criteria of selectivity are important for these catalysed reactions: substrate selectivity [9], regioselectivity [10], stereoselectivity (*endo*/*exo* [11] and *Z*/*E* [12] differentiation), enantioselectivity [13], *meso* differentiation [14] and prochiral recognition [15].

In many cases, the stereoselectivity of the enzyme used in water and in an organic solvent is the same [16, 17]; thus, complementary stereoisomers can be produced. If an enzyme prefers the *R* enantiomer of a chiral ester over the *S* ester, the *R* alcohol and the *S* ester can be obtained after a hydrolytic reaction. As the enzyme's stereochemical preference remains the same, transesterification in organic solvents will produce the *S* alcohol and *R* ester.

Theoretically, both reactions will stop at 50% conversion and will give both enantiomers with 100% enantiomeric excess [ee=(R–S)/(R+S)×100 for R>S], if the enzyme has an absolute stereoselectivity.

22.2.1.1 Lipolysis

Lipolysed milk fat was one of the first flavours produced with the help of enzymes. The original process was based on the controlled lipase-catalysed hydrolysis of cream [18]. For instance, *Mucor miehei* lipase possesses a high selectivity towards flavour-active short-chain fatty acids. Additionally, lipases that prefer long-chain fatty acids or lipases without particular preferences can be found. The free fatty acids produced can be isolated by steam distillation and further purified. Thus, it is possible to obtain pure short-chain fatty acids like butanoic, hexanoic, octanoic and decanoic acid.

Lipolysed milk fat products can serve as cream-like/butter-like flavouring agents [19].

22.2.1.2 Kinetic Resolution of Racemates

Stereoselectivity of lipases is often used to yield pure optically active flavour compounds from racemic precursors. This fact is important if one isomer of a molecule has more desirable properties than the other one.

For instance, (-)-menthol (*p*-menthan-3-ol) is one of the most important flavouring agents and is the major compound in natural peppermint oil. The characteristic peppermint odour and the typical cooling effect is limited to (-)-menthol. The other isomers do not show this refreshing effect. A racemic mixture of menthol holds an intermediate position: the cooling effect is still perceptible.

There are several biochemical and chemical processes for the resolution of a racemic mixture of menthol. Many microbiological lipases hydrolyse men-

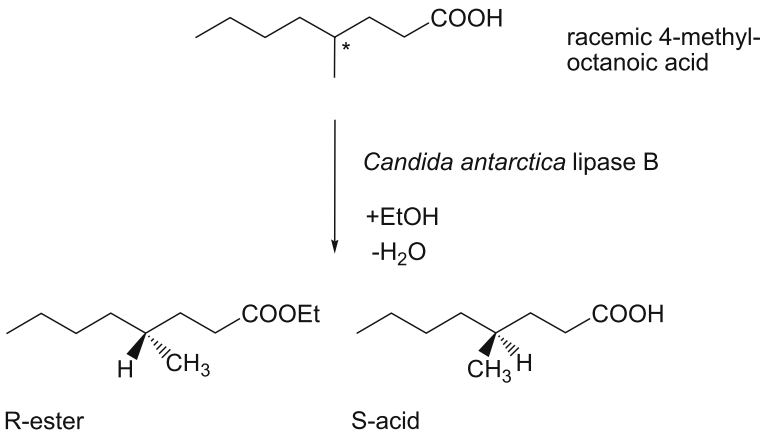
thyl esters and prefer the (-)-menthyl esters, whereas (+)-menthyl esters are not hydrolysed at all. This asymmetric hydrolysis of menthyl esters can be performed with lipases from *Penicillium*, *Rhizopus*, *Trichoderma* and various bacteria [20].

The enantioselective hydrolysis of racemic menthyl benzoate (industrially key compound) by recombinant *Candida rugosa* lipase LIP1 leads to optically pure l-(-)-menthol; ee>99% [21]. This pathway is part of a menthol synthesis developed by the flavour industry.

The resolution of the commercially available racemic *trans*-jasmonate to (-)-*trans*-jasmonate by microbial lipase has been described by Serra et al. [22].

Nozaki et al. [23] characterised the production of (+)-mesifuran [2,5-dimethyl-4-methoxy-3(2*H*)-furanone], an important flavour compound in arctic bramble, but which also occurs in strawberry and pineapple. After lipase-catalysed (*Candida antarctica*) enantioface-differentiating hydrolysis of the enol acetate, the pure optically active (+)-mesifuran could be obtained.

Kinetic resolution of branched-chain fatty acids has been reported recently by Franssen et al. [24]. With the help of immobilised *Candida antarctica* lipase B, racemic 4-methyloctanoic acid (responsible for sheep-like and goat-like flavours in sheep and goat milk and cheese, respectively) was esterified with ethanol. Only the *R* ester could be obtained, whereas (*S*)-4-methyloctanoic acid was not converted (Scheme 22.1).



Scheme 22.1 Kinetic resolution of racemic 4-methyloctanoic acid with *Candida antarctica* lipase B [24]

22.2.1.3 Catalysis in Organic Media

Lipase-catalysed esterification and transesterification reactions have a wide range of applications in the synthesis of aroma compounds.

The reaction conditions have a great influence on the enzyme-catalysed reactions in organic media and determine the reaction's yield and selectivity.

Enzymes require only a monomolecular water phase for their activity in organic solvents [25]; the pH of the water phase [26], temperature [27], type of solvent [28] and immobilisation techniques [29] will influence the reaction too.

Of course, the selection of the appropriate enzyme is fundamental because yield and selectivity of the enzymes vary extremely. For instance, *Candida rugosa* lipase will give high yields but has a low selectivity. In contrast, lipase from *Aspergillus niger* exhibits high selectivity [13].

The biotechnological production of flavour compounds is particularly focused on esters and lactones. Lipase from *Mucor miehei* is the most widely studied fungal lipase [30–35]. Esters of acids from acetic acid to hexanoic acid and alcohols from methanol to hexanol, geraniol and citronellol have been synthesised using lipases from *Mucor miehei*, *Aspergillus sp*., *Candida rugosa*, *Rhizopus arrhizus* and *Trichosporum fermentans* [32-37].

Methylbutanoates and methylbutyl esters are essential flavour compounds in fruit flavours; they can be produced biotechnologically as mentioned before. Chowdary et al. [33] have described the production of a fruit-like flavour: isoamyl isovalerate by direct esterification of isoamyl alcohol and isovaleric acid in hexane with the help of *Mucor miehei* lipase immobilised on a weak anion-exchange resin.

Synthesis of short-chain geranyl esters catalysed by esterase from *Fusarium oxysporum* in an organic solvent was reported by Stamatis et al. [39].

Large-scale synthesis of (*Z*)-3-hexenyl acetate in hexane with lipase, (*Z*)-3-hexenol and acetic acid was described by several authors [40–42]. (*Z*)-3-Hexenyl acetate has a fruity odour and shows a significant green note flavour. It can be produced using lipase from *Candida antarctica* immobilised on an acrylic resin [40, 41] or using immobilised lipase from *Mucor miehei* [42]. The conversion was reported to be about 90%.

An optimised enzymatic synthesis of methyl benzoate in an organic medium was reported by Leszczak and Tran-Minh [43]. Methyl benzoate is part of the aroma of some exotic fruits and berries. The ester has been produced by direct esterification of benzoic acid with methanol in hexane/toluene catalysed by lipase from *Candida rugosa*.

Gatfield et al. [44] reported in 2001 a method to produce natural ethyl (*E*,*Z*)-2,4-decadienoate, the impact compound of pear. Immobilised lipase from *Candida antarctica* is capable of transesterifying *Stillingia* oil in the presence of ethanol. By this process, a complex mixture of ethyl esters is generated. By fractional distillation, the ethyl ester of (*E*,*Z*)-2,4-decadienoate can be isolated from the mixture in a total yield of about 5% and with a high degree of purity. As only

natural precursors, physical and biological processes were used, the aroma compound obtained can be labelled as natural according to the legislation of the European Union.

In 2004, Ley et al. [45] showed a stereoselective enzymatic synthesis of *cis*-pellitorine [*N*-isobutyldeca-(2*E*,4*Z*)-dienamide], a taste-active alkamide naturally occurring in tarragon. The reactants were ethyl (*E*,*Z*)-2,4-decadienoate—the pear ester described before—and isobutyl amine. The reaction is catalysed by lipase type B from *Candida antarctica* (commercially available), which shows a remarkable selectivity towards the 2*E*,4*Z* ester. The yield was about 80%.

The biotechnological synthesis of lactones has reached a high standard. Besides microbial production, lactones can also be enzymatically produced. For instance, a lipase-catalysed intramolecular transesterification of 4-hydroxycarboxylic esters leads enantioselectively (ee>80%) to (*S*)-γ-lactones; the chain length may vary from C5 to C11 [13]. γ-Butyrolactone can be produced in that way with lipase from *Mucor miehei* [30].

The preparation of optically active δ-lactones is more difficult because of the lack of selectivity of most lipases.

22.2.2 Glycosidases (EC 3.2.1.X)

It is well-known that in plant tissues certain amounts of flavour compounds are bound as non-volatile sugar conjugates. Most of these glycosides are β-glucosides, but there are other glycones like pentoses, hexoses, disaccharides and trisaccharides too [46]. Acylated glycosides and phosphate esters have also been reported [47, 48]. Information about the analysis of glycosides can be found in the work of Herderich et al. [49].

Besides the structural elucidation of glycosides, research is focused on the application of glycosidases to liberate the aroma-active aglycons from their bound forms. The development of a continuous process of enzymatic treatment (simultaneous enzyme catalysis extraction) [50] opened the doors for the industrial large-scale production of aroma compounds from their non-volatile conjugates.

Major interest has been directed to wine. During winemaking, the grape's β-glucosidase is rapidly inactivated. Glucosidases from *Saccharomyces cerevisiae* and *Candida molischiana* have been suggested to solve this problem [51]. Nonetheless, many fungal glycosidases will not work properly, because they are inhibited by glucose, fructose, ethanol and the relatively low pH of wine. Some glycosidases from *Aspergillus* sp. (e.g. some β-apiosidases, α-arabinosidases and α-rhamnosidases) do not have these disadvantages. The formation of these enzymes can be induced by the presence of the respective glycoside; their use has been patented for application to grape must [52]. Cabaroglu et al. [53] have given a comprehensive overview of wine flavour enhancement by the use of fungal glycosidases and have shown that enzymatically treated wine was preferred in sensory analyses.

Sensory quality of food can be improved by synergistic action of monoglycanases, oligoglycanases and polyglycanases. A process for the production of vanilla extracts involving the treatment of crushed green vanilla beans with enzymatic preparations that degrade plant cell walls and the glucosidic precursor together has been patented [54]. Similarly, a cellulase possessing glucosidase side activity has been reported to liberate benzaldehyde from its bound form during the processing of peach [55].

Raspberry ketone [4-(4′-hydroxyphenyl)-butan-2-one], the impact compound found in raspberries, can be obtained by enzymatic reactions: The first step is the β-glucosidase-catalysed hydrolysis of the naturally occurring betuloside to betuligenol. The latter can be transformed into raspberry ketone by microbial alcohol dehydrogenase (Scheme 22.2) [56].

Conjugates of flavour compounds were also found in milk: phenols can be liberated by β-glucuronidase, arylsulfatase and acid phosphatase from their respective precursors [57].

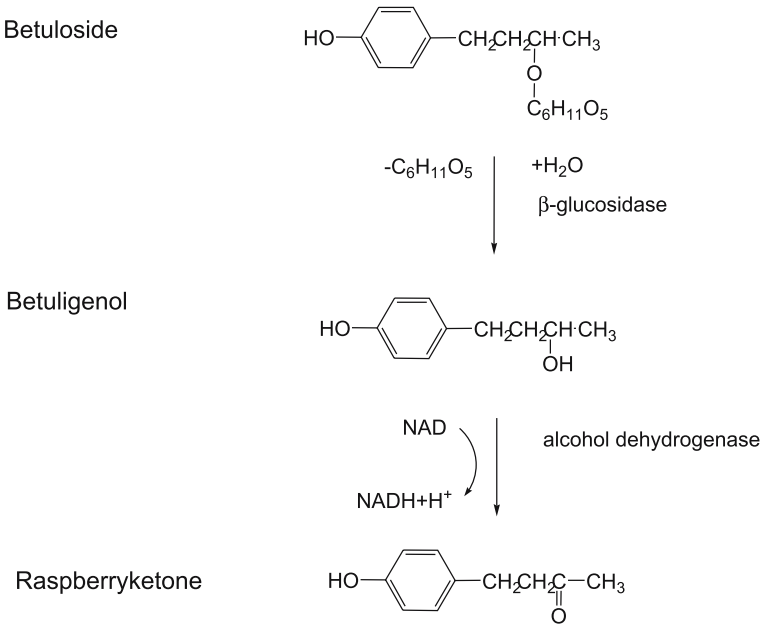
Besides the liberation of bound flavour compounds, the creation of these conjugates is becoming more and more important, especially for convenience food. A bound, non-volatile aroma compound allows the slow liberation of the flavour upon heating. These slow-release compounds are produced with the help of glucosidases in a reversed hydrolysis reaction. For instance, the production of geranyl glucoside was described by de Roode et al. [58] and Franssen et al. [24]. Glycosyl transferases are also able to produce glycosides, but they are more complicated to handle than glucosidases [24].

There are synthetic acetal derivatives of flavour-active aldehydes like benzaldehyde and cinnamaldehyde [59].

As the chemical synthesis of glycosides is cumbersome, biotechnological transglycosidation using glycosidases is attracting more and more attention [60].

22.2.3 Flavorzyme®

Flavorzyme® is a commercially available proteolytic enzyme preparation by Novo Nordisk Bioindustrials. It can be used to obtain a meat-like process flavouring from defatted soybean meal. With the help of aroma extract dilution analysis, Wu and Cadwallader [61] showed in their study of 2002 the presence of key aroma compounds of roasty, meat-like aroma in the enzymatically hydrolysed and heated hydrolysed protein, e.g. maltol, furaneol, methanethiol and furanthiol derivatives.



Scheme 22.2 Enzymatic production of raspberry ketone from betuloside [56]

22.3 Oxireductases

Many enzyme-catalysed redox processes include the transfer of the equivalent of two electrons by one two-electron step or two one-electron steps. The latter is considered as a radical process involving the use of cofactors like flavin, quinoid coenzymes or transition metals.

The two-electron process is either a hydride transfer or a proton abstraction followed by two-electron transfer.

22.3.1 Horse Liver Alcohol Dehydrogenase (EC 1.1.1.1)

Horse liver alcohol dehydrogenase is able to oxidise primary alcohols—except methanol—and to reduce a large number of aldehydes. Aqueous solution or organic solvents can be used [62]. As there are no new developments concerning this enzyme, the reader is referred to the review of Schreier [1].

22.3.2 Lipoxygenase (EC 1.13.11.12)

Lipoxygenase (LOX) is a non-haem, iron-containing dioxygenase that catalyses the regioselective and enantioselective dioxygenation of unsaturated fatty acids containing at least one (*Z*,*Z*)-1,4-pentadienoic system. For instance, LOX from soy converts linoleic acid to the (*S*)-13-hydroperoxide [1].

It is supposed that the catalytic mechanism proceeds through a free-radical intermediate which reacts directly with oxygen or an organic iron intermediate [63]. The three-dimensional protein structure of the native form of LOX isoenzyme L-1 from soybean has already been described [64, 65].

LOX is an important factor in the large-scale use of plant enzymes for the production of natural "green note" aroma compounds, a group of isomeric C6 aldehydes and alcohols [66].

In nature, the green notes are produced after the destruction of the plant's tissue (leaves, fruits or vegetables). Destruction of the cell wall leads to a cascade of enzyme-catalysed reactions; polyunsaturated fatty acids with the diene system described before are converted into hydroperoxides by LOX catalysis. The hydroperoxide lyase cleaves the hydroperoxides; in the whole cascade, oxireductases are involved too. The biotechnological large-scale production of natural green notes follows the natural pathway.

A patented process for the production of green notes applying baker's yeast for in situ reduction of enzymatically produced aldehydes [67, 68] has been called into question regarding the effective production of (*Z*)-3-hexenol. According to Gatfield's report [69] the isomerisation of (*Z*)-3-hexenol to (*E*)-2-hexenal is a very fast process. The latter undergoes facile conversion to hexanol. Beside this, baker's yeast can add activated acetaldehyde to (*E*)-2-hexenal, forming 4-octen-2,3-diol.

At present, there are some patents concerning the production of green notes by recombinant guava 13-hydroperoxide lyase expressed in *Escherichia coli* [70, 71] and *Cucumis melo* hydroperoxide lyase; the latter yields a mixture of C6 and C9 compounds [72].

Fungal LOXs exhibit different regioselectivity from LOX from higher plants; they catalyse the formation of 10-hydroperoxides from linoleic and linolenic acid by dioxygenation. Hydroperoxide lyase and subsequent enzymes in the damaged fungal cells are able to form the typical volatile mushroom aroma substances, including the impact compound (*R*)-1-octen-3-ol. The latter can be produced industrially by feeding the mycelia with linoleic acid [73, 74].

It is a well-known fact that soybean LOX is able to cooxidise plant pigments, such as carotenoids and chlorophyll in the presence of linoleic acid. The hypothesis of a free-radical mechanism has been supported by stereochemical studies of the unselective formation of epoxides during LOX-catalysed cooxidation [75].

A pathway for the production of α-ionone and β-ionone by LOX-catalysed cooxidation of carotenes has been described [76].

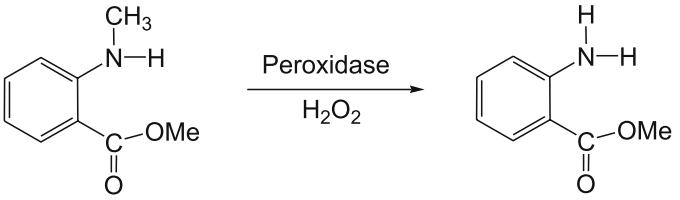
22.3.3 Peroxidases (EC 1.11.1.X)

22.3.3.1 Soybean Peroxidase

The production of methyl anthranilate, which has a fruity odour, by enzymatic N-demethylation of methyl *N*-methyl anthranilate (Scheme 22.3.) has been reported by van Haandel et al. [77]. Self-prepared soybean peroxidase (haem-based enzyme) preparation and H_2O_2 were used.

The reaction product can be labelled as natural if the methyl *N*-methyl anthranilate used has a natural origin, e.g. methyl *N*-methyl anthranilate extracted from citrus leaves.

An alternative method for the production of methyl anthranilate with the help of *Bacillus megaterium* was recently reported by Taupp et al. [78]; the latter pathway resulted in higher yields of methyl anthranilate.



Scheme 22.3 Production of methyl anthranilate by enzymatic N-demethylation of methyl *N*-methyl anthranilate [77]

22.3.3.2 Horseradish Peroxidase (EC 1.11.1.7)

The haem peroxidases are a superfamily of enzymes which oxidise a broad range of structurally diverse substrates by using hydroperoxides as oxidants. For example, chloroperoxidase catalyses the regioselective and stereoselective halogenation of glycals, the enantioselective epoxidation of distributed alkenes and the stereoselective sulfoxidation of prochiral thioethers by racemic arylethyl hydroperoxides [62]. The latter reaction ends in (*R*)-sulfoxides, (*S*)-hydroperoxides and the corresponding (*R*)-alcohol, all in optically active forms.

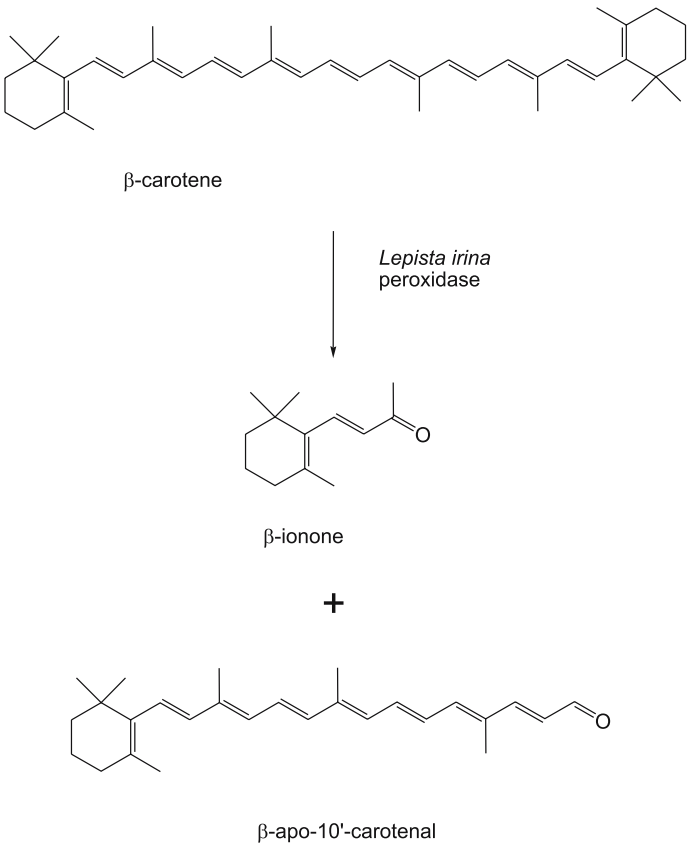
Horseradish peroxidase catalysed kinetic resolution of racemic secondary hydroperoxides has been described by Adam et al. [79]. The reaction yields (*R*)-hydroperoxides up to ee>99% and (*S*)-alcohols up to ee>97%. Optically active hydroperoxides as potential stereoselective oxidants can be obtained by this process.

As horseradish peroxidase is relatively expensive and possesses only little thermostability, the industrial application of horseradish peroxidase is limited [77].

22.3.3.3 *Lepista irina* Peroxidase

In 2003, Zorn et al. [80] discovered a fungal peroxidase from *Lepista irina*—a valued edible fungus—that cleaved *β*,*β*-carotene to flavour-active compounds. According to the authors, the cleavage of *β*,*β*-carotene to aroma compounds by a fungal peroxidase had not been reported before.

It was found that extracellular liquid of the fungus can degrade *β*,*β*-carotene to *β*-cyclocitral, dihydroactinidiolide, 2-hydroxy-2,6,6-trimethylcyclohexanone, *β*-apo-10´-carotenal and *β*-ionone; the last two compounds are the main prod-



Scheme 22.4 Cleavage of *β*-carotene by *Lepista irina* peroxidase [80]

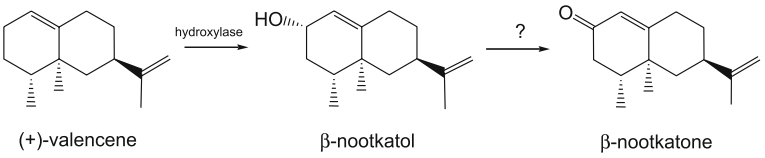
ucts (Scheme 22.4). The key enzyme catalysing the oxidative cleavage was isolated and characterised.

As there is great interest from the detergent, food and perfume industry in the potent aroma compounds formed by carotenoid breakdown, and as the *β*-ionone obtained can be labelled as natural aroma—if natural carotenoids are used—this cleavage reaction might have a high potential.

22.3.4 Laccase (EC 1.10.3.2)/Germacrene A Hydroxylase

Laccase, a group of multi-copper proteins of low specificity, acting on both *o*-quinols and *p*-quinols and often on aminophenols and phenylenediamine, is used for the biotechnological production of nootkatone, the impact compound of grapefruit. Huang et al. [81] described a process for the laccase-catalysed oxidation of valencene to nootkatone; they used whole microorganisms with laccase activity—such as from *Botrytis cinera*—but they reported a process with isolated laccase too. The first step of the reaction is the formation of valencene hydroperoxide, which undergoes a non-enzymatic degradation to nootkatone. The yield was about 60%.

Franssen et al. [24] pointed out an alternative method of production of nootkatone from valencene catalysed by (+)-germacrene A hydroxylase, an enzyme of the cytochrome P450 monooxygenase type that was isolated from chicory roots. In general, this enzyme appeared to accept a broad range of sesquiterpenes and hydroxylates exclusively at the side-chain's isopropenyl group. Valencene is an exception: it was not hydroxylated at the side chain, but *β*-nootkatol was formed in the first step (Scheme 22.5); it is not yet clear if the second step is enzyme-catalysed.



Scheme 22.5 Production of nootkatone from valencene catalysed by (+)-germacrene A hydroxylase [81]

22.3.5 Microbial Amine Oxidases (EC 1.4.3.X)

Amine oxidase from *Aspergillus niger* and monoamine oxidase from *Escherichia coli* can be used for the oxidative deamination of amines, forming the corre-

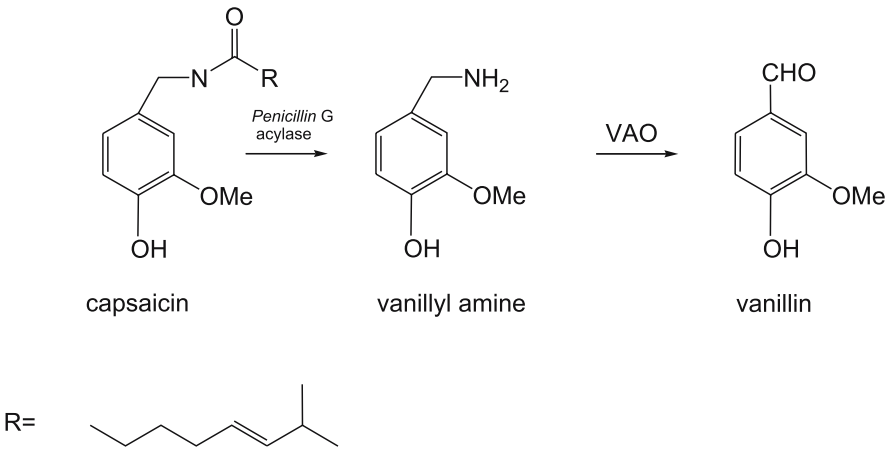
sponding aldehydes, hydrogen peroxide and ammonia. Using these enzymes, Yoshida et al. [82] described a pathway for the production of vanillin (4-hydroxy-3-methoxy-benzaldehyde).

Vanillylamine [(4-hydroxy-3-methoxy-phenyl)methylamine] is the substrate of choice for the formation of vanillin with the help of amine oxidase. It can be obtained by cleavage of capsaicin (*N*-[(4-hydroxy-3-methoxy-phenyl)methyl]-8-methyl-6-nonenamide) isolated from pepper and capsicum [83]. As natural vanillin extracted from beans of *Vanilla planifolia* is rare and extremely expensive, this pathway for the production of natural vanillin is regarded to have a great potential. The vanillin obtained by the process can be labelled as natural if the cleavage of capsaicin is performed enzymatically.

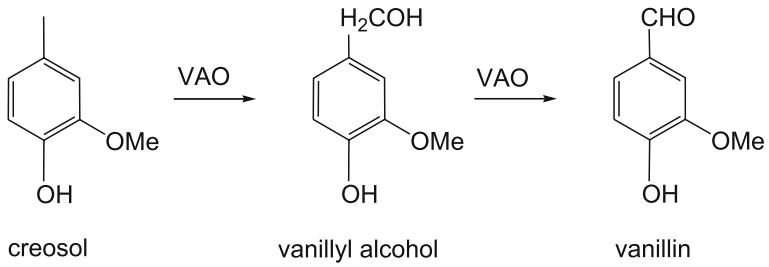
22.3.6 Vanillyl Alcohol Oxidase (EC 1.1.3.38)

Vanillyl alcohol oxidase (VAO) is a flavoenzyme from the ascomycete *Penicillium simplicissimum* that converts a broad range of 4-hydroxybenzyl alcohols and 4-hydroxybenzylamines into the corresponding aldehydes. This large substrate specificity makes it possible to obtain vanillin from two major pathways.

As VAO is able to perform an oxidative deamination of capsaicin-derived vanillyl amine, vanillin can be produced by the pathway described in the previous subsection. Van den Heuvel et al. [83] pointed out this biocatalytic route of synthesis in 2001 using penicillin G acylase to obtain vanillyl alcohol from natural capsaicin (Scheme 22.6). As the vanillin obtained can be labelled as natural,



Scheme 22.6 Oxidative deamination of capsaicin-derived vanillyl amine and formation of vanillin [83] *VAO* vanillyl alcohol oxidase



Scheme 22.7 Production of vanillin from creosol by two enzymatic reactions [83]

the enzymes used do not require expensive cofactors and the enzymes can be produced on a large scale, this bi-enzymatic process could be promising.

The second pathway using VAO reported by van den Heuvel et al. [83] is the VAO-catalysed oxidation of vanillyl alcohol to vanillin. Vanillyl alcohol is not very abundant in nature but can be generated by VAO-catalysed conversion of creosol (2-methoxy-*p*-cresol). As creosol can be found in creosote obtained from heating wood or coal tar, the feedstock for this pathway is very abundant.

The process comprises two steps: the conversion of creosol to vanillyl alcohol and the oxidation of the alcohol to vanillin (Scheme 22.7). Interestingly, these two steps are catalysed by the same enzyme, i.e.VAO.

In 2004, van den Heuvel et al. [84] described in another study the characteristics of VAO and pointed out details of the reaction's mechanism.

22.4 Transferases

22.4.1 Cyclodextrin Glucanotransferase (EC 2.4.1.19)

In 2002, Do et al. [85] proposed a pathway for the enzymatic synthesis of (-)-menthyl α-maltoside and α-maltooligosides from (-)-menthyl α-glucoside using cyclodextrin glucanotransferase obtained from *Bacillus macerans*. The reaction can be performed in a reactor containing (-)-menthyl α-glucoside, the enzyme and soluble starch; the yield was about 80%:15% (-)-menthyl α-maltoside and 65% (-)-menthyl α-maltooligosides, respectively. Treatment of the starch with α-amylase can raise the proportion of (-)-menthyl α-maltoside.

At first, (-)-menthyl α-maltoside has a bitter and sweet taste, but after a few minutes, the refreshing flavour occurs. It has the potential to become a slow-release aroma compound in foods or cigarettes because it possesses higher solubility in water and has a lower tendency to sublimate.

22.5 Lyases

22.5.1 D-Fructose-1,6-biphosphate Aldolase (EC 4.1.2.13)

The formation of C–C bonds by aldol condensation is a very useful method in synthesis. Besides the chemical synthesis, aldolases can be used to perform this reaction. The reaction yields a stereoselective condensation of an aldehyde with a ketone donor.

In nature, four complementary aldolases can be found in the carbohydrate metabolism. They show different stereoselectivity and this broad range of enzymes makes it possible to fulfil a large variety of synthetic tasks. In biotechnology, Furaneol® (2,5-dimethyl-4-hydroxy-2*H*-furan-3-one) can be produced from fructose-1,6-biphosphate with the help of a three-step enzymatic process involving fructose-1,6-bisphosphate aldolase (rabbit muscle aldolase). The first step is the aldolase-catalysed cleavage of the sugar biphosphate to dihydroxyacetone phosphate and glyceraldehyde phosphate. The latter is isomerised by a coimmobilised triose phosphate isomerase to obtain dihydroxyacetone phosphate, which is the substrate for the aldolase-catalysed aldol condensation with d-lactaldehyde. The condensation's product, 6-deoxyfructose phosphate, can be easily converted to Furaneol® [86].

In spite of the intensive effort regarding the biosynthesis of Furaneol® (including the detection of some important enzymes), the biosynthesis in plants is still not fully understood [87].

22.5.2 Sesquiterpene Synthase (EC 4.2.3.9)

In the last few years, sesquiterpene synthase from different plants has raised attention. In 2004, Schalk and Clark [88] described a process (patented by Firmenich, Switzerland) that makes it possible to obtain sesquiterpene synthase and to produce various aliphatic and oxygenated sesquiterpenes from farnesyl diphosphate. For instance, valencene can be obtained in this way.

One year later, Schalk [89] described a process for cloning sesquiterpene synthases from patchouli plants (*Pogostemon cablin*) and the enzyme-catalysed terpenoid production. Various sesquiterpenes can be obtained by this method, for instance patchoulol and other germacrene-type sesquiterpenes.

22.6 Conclusion

Thanks to the intense research during the last 20 years, flavour biotechnology is an integrated part of industrial aroma production, in which enzyme-catalysed

reactions are an alternative to microbial-based processes. Besides the production of aroma, enzymes can also be used to refine aroma of cheese or wine.

The great advantage of enzymes is their stereoselectivity and the ability to produce "natural" aroma if "natural" substrates are available.

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