# Bioflavours and fragrances via fungi and their enzymes

**Erick J. Vandamme**[*]

Laboratory of Industrial Microbiology and Biocatalysis, Department of Biochemical and Microbial Technology, Faculty of Agricultural and Applied Biological Sciences, Ghent University, Coupure links 653, B-9000 Ghent, Belgium; e-mail: erick.vandamme@rug.ac.be

Many fungi and yeasts have been found to produce *de novo* odorous compounds. *Ceratocystis* species and the yeasts *Kluyveromyces lactis* and *Sporidiobolus salmonicolor* produce a wide range of terpenes and lactones with fruity or floral flavours. The yeast *Williopsis saturnus* synthesizes *de novo* fruity ester flavours (i.e. volatile branched acetates); their yield can be improved by feeding fusel oil as a cheap source of precursor branched alcohols to the fermentation process. *Geotrichum klebahnii* also produces a broad spectrum of ethylesters of branched carboxylic acids, generating a pleasant fruity flavour. Also, precursor speciality fatty acids and PUFA's can be converted by fungi (such as *Penicillum* sp. and *Botryodiplodia* sp.) into flavour compounds, that provide "green notes", mushroom flavour, fruity lactones and cheese-flavoured methylketones. Similarly, a two step fungal process has been developed, whereby *Aspergillus niger* tranforms ferulic acid into vanillic acid, which basidiomycetes such as *Pycnoporus cinnabarinus* or *Phanerochaete chrysosporium* can further convert into vanillin. Furanone-flavours occur in many fruits, but have also been detected in microbial cultures. In this context, the soy sauce yeast *Zygosaccharomyces rouxii* forms the DMHF-furanone compound from glucose, when fed with fructose-1,6-biphosphate. Apart from precursored fermentation processes, enzymatic systems are also being developed to produce flavours i.e. yeast alcohol dehydrogenase can convert 1-phenyl-2-propanone into (S)-1-phenyl-2-propanol; *in vitro* co-enzyme regeneration often remains a bottleneck. Yeasts such as *Torulopsis bombicola* and *Candida tropicalis* can convert fatty acids or alkanes into musk-fragrance precursors. These examples indicate that interdisciplinary cooperation between microbiologists, biochemists, organic chemists and bioprocess engineers is needed to develop interesting laboratory findings into economic bioflavour production processes.

**Key words:** bioflavour, enzymes, furanones, fungi, fruity esters, green notes, vanilla, musk fragrance, terpenes, yeast

## Introduction

Since time immemorial, man has unwittingly used microorganisms to produce flavours, especially when preparing fermented foods and drinks, the original benefit being the increased shelf life of such products. It was only

---

[*]Corresponding author: e-mail: erick.vandamme@rug.ac.be

around the turn of the 20th century, that the relationship between the typical desirable flavour of fermented foods and beverages, and the microorganisms involved became recognized. In most cases, a typical mixed microbiota was responsible for a desirable but complex flavour formation i.e. in cheese, yoghurt, kefir, sourdough, gueuze beer, sauerkraut, kimchi, miso, tempeh, ontjorn and soy sauce. Undesirable off flavours were then caused by spoilage or contaminant microbiota.

Further analysis and optimisation of such food fermentations, led to the study of pure microbial strains as to their capacity to produce specific single flavour molecules either *de novo* or by converting an added substrate/precursor molecule.

In only a few cases, detailed research has led to the identification of the biochemical pathways involved, but much work still lays ahead here; similarly several enzymes/enzyme systems have been characterised and are now being exploited for enzymatic flavour synthesis.

## Chemical, nature-identical and natural flavours

Flavours and fragrances nowadays have a wide application in the food, feed, cosmetic, chemical and pharmaceutical sector. Many flavour compounds on the market are still produced via chemical synthesis or via extraction from plant and animal sources; however, a rapid switch towards the bio-production and use of flavour compounds of (micro) biological origin - bioflavours - is observed. Reasons are among others, the fact that chemical synthesis results often in an environmentally unfriendly production processes and in undesirable racemic mixture compounds. Furthermore, the consumer has developed a "chemophobia"-attitude towards chemical or synthetic (even nature-identical) compounds, especially when related to his food and home-care products (Cheetham, 1993, 1997).

Products that occur in nature but are now produced via a chemical (a non-natural) process are called "nature-identical"; this mode of production is no longer accepted as consumer friendly.

Up to now, certain plant and animal sources remain an important source of bioflavours, but these bio-active compounds are often present in minor quantities, making extraction, isolation and formulation very expensive, or they are found only in exotic (plant) species.

The other bio-route for flavour synthesis is based on *de novo* microbial processes (fermentation) or on bioconversions of natural precursors with microbial cells or enzymes (biocatalysis). Biotechnological processes usually require less damaging process conditions to the environment and yield the desirable enantiomeric flavour compound. A bottleneck often still is a lack of

154

knowledge about the biochemical pathways, the enzymes and the metabolic (de)regulation involved, to obtain high yields. Nevertheless, bioflavours/biofragrances appeal to many sectors and represent already a high market value (Berger, 1995; Gatfield, 1997; Krings and Berger, 1998).

According to USA as well as European regulations, natural flavours include products, obtained through microbial or enzymatic processes, as long as the precursor/raw material be natural and obtained via physical or bio-processes and that the precursor and product can be found in nature or are part of traditional foods.

Physical processes for obtaining natural flavours are extraction, distillation, concentration and crystallisation, i.e. from animal sources (beef, chicken and seafood) or plant sources (spices, mushroom, citrus, fruits and mints). A wide range of microorganisms (bacteria, fungi, yeasts) are known to produce via *de novo* synthesis flavour compounds from simple nutrients i.e. sugars and alcohols. Bulk flavour compounds belonging to this category include vinegar, monosodiumglutamate, 5'-GMP and 5'-IMP-nucleotides, organic acids (L-lactic acid, citric acid and propionic acid) and yeast extracts. These will not be discussed in this paper. The focus will be on speciality flavours; a review has been published by the authors' group in 1992 (Janssens *et al.* 1992) and several updates have appeared since then (Hagedorn and Kaphammer, 1994; Gatfield, 1997; Krings and Berger, 1998).

A summary will be given here of the current state of art of microbial and enzymatic bioflavour synthesis, with emphasis on fungi and yeasts able to produce odorous compounds.

### *De novo*-flavour biosynthesis via fungal fermentation

### *Floral and fruity lactones*

Several filamentous fungi are able to produce *de novo* odorous compounds, including floral flavours. *Ceratocystis* sp. are well-known to produce a wide range of terpenes, with fruity or floral odour (Fig. 1). *Trichoderma viride* forms efficiently the coconut flavoured lactone, 6-pentyl-α-pyrone (6-PP); in an integrated fermentation process, 6-PP can be continuously removed by pervaporation over a selective membrane, since it inhibits growth upon accumulation in the broth (Häusler and Munch, 1997; Bleumke and Schrader, 2001; Palomares *et al.*, 2001).

Yeasts such as *Kluyveromyces lactis* also produce *de novo* fruity, floral flavour-terpenes such as citronellol, linalool and geraniol. Takahara *et al.* (1973) found that the yeast *Sporobolomyces odorus* (now *Sporidiobolus*
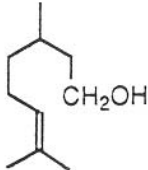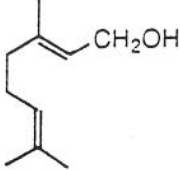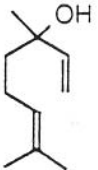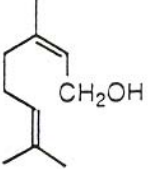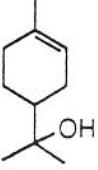
| Terpene | Structure | Sensorial description |
| --- | --- | --- |
| citronellol | | fresh, rose-like bitter taste |
| geraniol | | sweet, rose-like, fruity |
| linalol | | sweet, fresh, citrus, floral |
| nerol | | sweet, floral, rose-like, slightly bitter |
| α-terpineol | | sweet, floral, fruity when diluted |

**Fig. 1.** Terpenes produced by the fungus *Ceratocystis variospora*.

*salmonicolor*) produces *de novo* - in low yields though - flavour-lactones, such as the peach-smelling compound γ-decalactone (4-decanolide) and 4-hydroxy-cis-6-dodecenoic acid-γ-lactone.

In most cases the *de novo* fermentation processes can be boosted by supplying to the fungal culture a limiting intermediate or precursor molecule. A well-studied and industrial example here is the microbial conversion of

ricinoleic acid (12-hydroxy-$C_{18}$:1), the main constituent of castor oil, via partial β-oxidation into the peach-like lactone aroma, γ-decalactone (which is a chiral molecule) by yeasts such as *Sporidiobolus salmonicolor* and *Yarrowia lipolytica*. Yields over 10 g/l have been reported (Fig. 2). Quite often, undesirable side products are formed. A trick, which sometimes can be applied, is to metabolise "away" the side product or intermediate from the fermentation or extraction liquid with another microorganism. For example, during the above mentioned γ-decalactone process from ricinoleic acid, a side product 3-hydroxy-γ-decalactone is formed, which is co-extracted; during subsequent distillation, the side product is converted into 3,4 unsaturated γ-decalactone, and then stereoselectively reduced into the desirable γ-decalactone by *Saccharomyces cerevisiae* yeast. The R-enantiomer occurs in peaches and most other fruits, the S-enantiomer in mango-varieties. Chemical synthesis yields the undesirable racemic mixture. The annual potential market for natural γ-decalactone is estimated at 10 tons; this corresponds to a fermentation capacity need of about 2.500 $m^3$ at current yields.
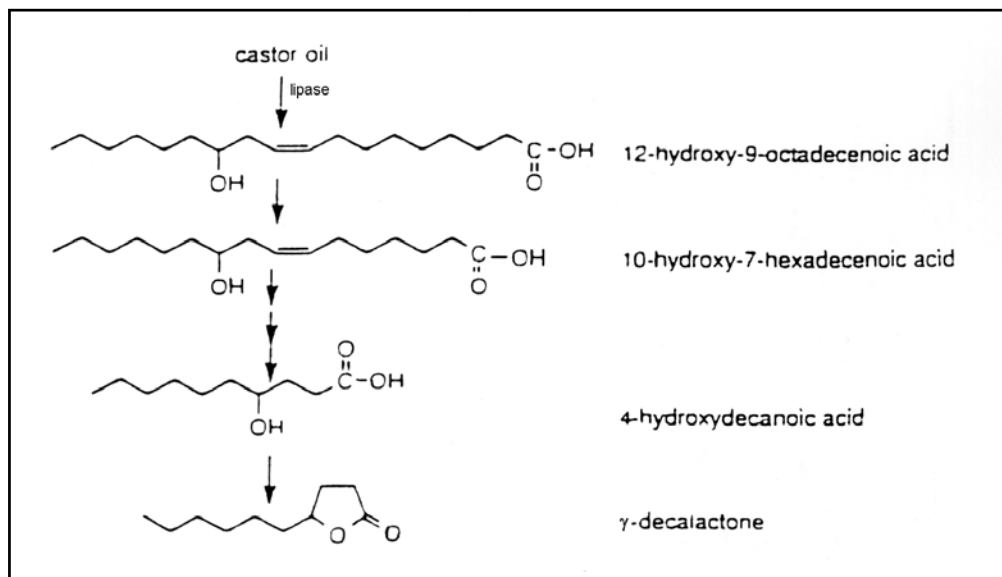


**Fig. 2.** Production of 4-decalactone through bioconversion of castor oil.

## *Fruity esters*

We have found several yeasts capable of producing *de novo* large amounts of fruity ester-flavours (Janssens *et al*., 1987; 1989; 1992). *Williopsis saturnus* var. *mrakii* synthesizes important levels of volatile branched acetates,

especially 3-methylbutyl-acetate, the character impact compound of banana aroma. Biochemically, the amino acids valine, leucine and isoleucine are metabolised by the yeast into the intermediate branched alcohols, isobutanol, 3-methylbutanol and 2-methylbutanol, respectively. Via action of alcoholacetyltransferases, the corresponding volatile-branched acetates, isobutylacetate, 3-methylbutylacetate and 2-methylbutylacetate are then formed. The fungus is able to convert added branched alcohols into the corresponding fruity acetates, thereby drastically improving the yield. As a natural source of these branched alcohols, fusel oil (a cheap by-product of the rectification of fermentation alcohol) was used. Due to its toxicity, fusel oil has to be added at low levels and after the active growth phase. A clear selectivity in ester formation was observed in the sense that 3-methylbutanol was esterified much faster and in higher yield (± 90%) into 3-methylbutylacetate (Fig. 3). The influence of fermentation parameters such as pH and aeration upon biomass formation and the esterification were investigated up to the 20 L laboratory fermentor scale. The process consists of an active growth phase, followed by bioconversion of the added fusel alcohols during the stationary phase. Using the aeration-air of the fermentation, the acetate-esters which are very volatile, are stripped from the fermentation broth, adsorbed on activated coal at the exhaust of the fermentor, and subsequently recovered by solvent extraction. Another yeast, *Geotrichum klebahnii* produces *de novo* a broad spectrum of ethyl esters of branched carboxylic acids, generating a pleasant fruity flavour. When supplied with isoleucine, especially ethyl-2-methylbutyrate was formed (Janssens *et al*., 1989).

**Precursored fungal fermentation for bioflavours**

***Aromatic phenols as substrate for vanillin-flavour formation by fungi***

Vanillin is now produced by chemical synthesis from guaiacol (at 12000 tons per year and 13.5 US $/kg) or via extraction from vanilla beans (natural vanilla content 2% w/w), (at 20 tons per year and 3200 US $/kg). The high price of natural vanillin and the trends towards natural flavours has driven the search for microbial processes for natural vanillin production (Priefert *et al*., 2001). This requires a natural precursor such as eugenol or ferulic acid, which are present in plants. White rot fungi are known to metabolise ferulic acid into vanillic acid and vanillin in low yields (Fig. 4). A two-step process has been developed, whereby *Aspergillus niger* transforms ferulic acid into vanillic acid, which basidomycetes such as *Pycnoporus cinnabarinus* or *Phanerochaete chrysosporium* can further convert into vanillin (500 mg/l) (Stentelaire *et al*., 2000). Fermentation process optimisation resulted in levels over 1g/l. Above

this level, vanillin is highly toxic for the producer cells; *in situ* adsorption on Amberlite XAD-2 resins reduced the toxicity via entrapping the produced vanillin and further increased the yield up to 1.57 g/l.
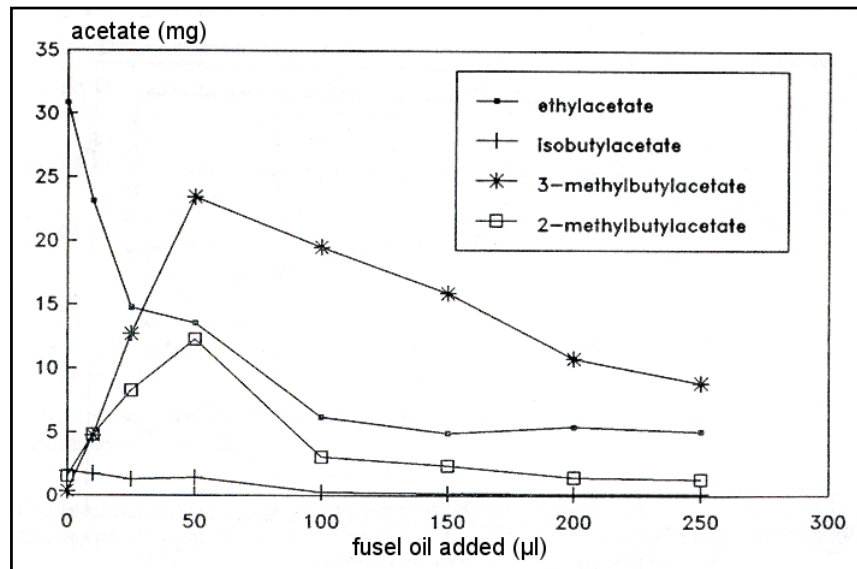


**Fig. 3.** Influence of fusel oil addition on flavour-acetate formation by *Williopsis saturnus* var. *mrakii* cultures. Addition was started only after 30 hours of fermentation; the volatile acetates were collected on activated carbon between 30 and 72 hours of fermentation (Janssens *et al.*, unpublished results).

### *Furanone-flavours via yeast*

Furaneol®, 2,5-dimethyl-4-hydroxy-3(2H)-furanone (DMHF) is an aroma, exhibiting strawberry flavour in dilute solutions and caramel-like flavour in concentrates (Fig. 5). Furanones occur in many fruits (e.g. pineapple, strawberries, mangoes, raspberries), but also in certain microbial cultures and in soy sauce.

It is also formed by the chemical reaction of sugars with amines during the Maillard reaction, and it can be chemically prepared, starting from specialty sugars such as L-rhamnose and L-fucose. The deoxysugar, L-rhamnose can be liberated from the bitter citrusglycosides, naringin and hesperidin, via rhamnosidase, an activity present in commercial *Aspergillus* pectinase preparations. L-fucose (6-deoxy-galactose) is a major constituent deoxy-sugar moiety of the capsular exopolysaccharide (EPS) of certain *Clavibacter* and *Klebsiella* strains. High yielding *Clavibacter*-EPS fermentation processes have been developed by the authors; the purified EPS, called clavan, can then
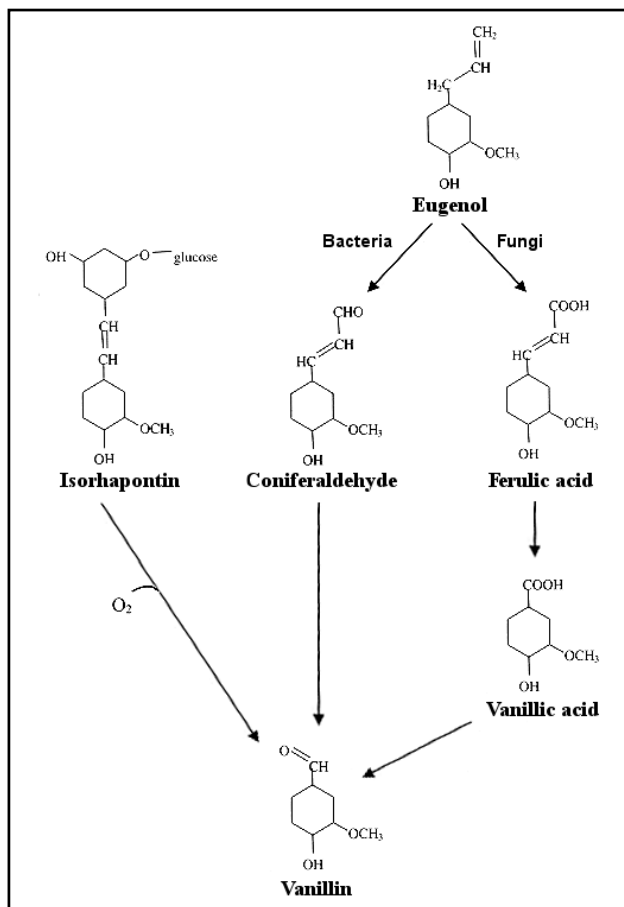
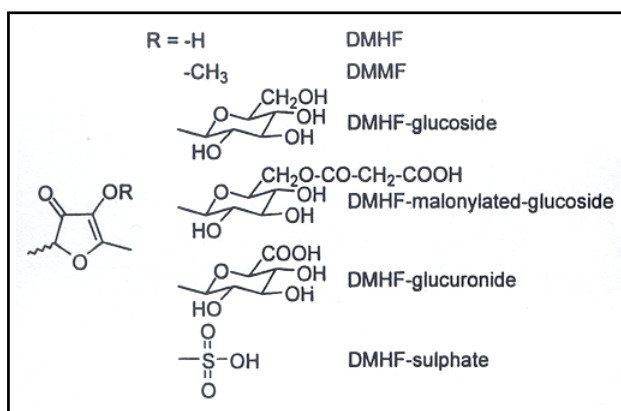**Fig. 4.** Routes for the bioproduction of vanillin.



**Fig. 5.** Chemical structures of naturally occurring 2,5-dimethyl-4-hydroxy-3(2H)-furanones (DMHF).

160

chemically or enzymatically be hydrolysed, to deliver free L-fucose (Vanhooren and Vandamme, 1999).
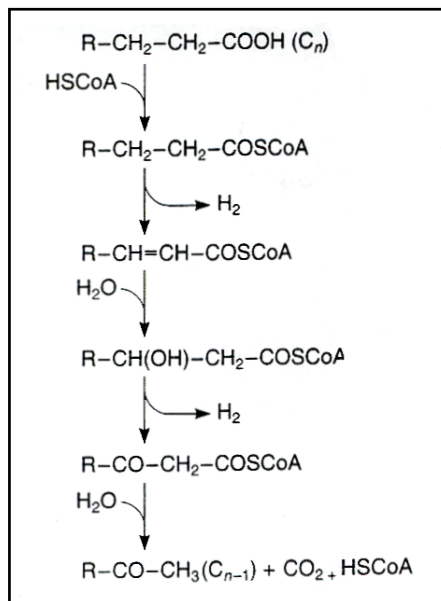
Recently, it has been shown that the soy sauce yeast *Zygosaccharomyces rouxii* is able to form DMHF, especially when the medium is supplied with D-fructose-1,6-biphosphate (FBP) and glucose; the cheap availability of FBP could help here to develop a microbial rather than a chemical process (Dahlen *et al.*, 2001).

### *Fatty acids and PUFA's as substrate for flavour formation*

Several valuable flavours and fragrances can be produced by fungi from speciality fatty acids, added as precursors, including compounds that provide "grassy green notes", mushroom flavours, specific lactones and methylketones (Feron *et al.*, 1996).

Methylketones (2-alkanones) derive from medium length fatty acids, and confer strong cheese associated flavours; they are the basis for flavour development in Roquefort, Camembert and Stilton cheese. Via an incomplete β-oxidation route and specific enzymes such as 3-ketoacyl CoA-thioester hydrolase, methylketones are formed by the cheese fungi (e.g. *Penicillium roquefortii*), which are one carbon shorter than their precursor 6- to 12 carbon fatty acid, such as 2-pentanone, 2-heptanone, 2-nonanone and 2-undecanone (Fig. 6). A large scale process uses the conidia of *Penicillium roquefortii* as the enzyme source on lipase-treated milk fats as fatty acid source.

**Fig. 6.** Cheese associated flavours (methylketones) formed by *Penicillium roquefortii* from medium length fatty acids.



$$R-CH_2-CH_2-COOH \ (C_n)$$
$$HSCoA \searrow \downarrow$$
$$R-CH_2-CH_2-COSCoA$$
$$\downarrow \rightarrow H_2$$
$$R-CH=CH-COSCoA$$
$$H_2O \searrow \downarrow$$
$$R-CH(OH)-CH_2-COSCoA$$
$$\downarrow \rightarrow H_2$$
$$R-CO-CH_2-COSCoA$$
$$H_2O \searrow \downarrow$$
$$R-CO-CH_3 \ (C_{n-1}) + CO_2 + HSCoA$$

Starting from α-linolenic acid (C18:3; found in linseed oil), the fungal plant pathogen *Botryodiplodia theobromae* can also form methyl(+)-7-iso-jasmonic acid, which displays a sweet floral, jasmine-like odour. A complex pathway – which occurs normally in plants – involves lipoxygenase (a dioxygenase that acts on cis-cis- pentadiene units of PUFA's), allene oxide synthase, cyclase enzymes, followed by β-oxidation steps and double bond reduction (Fig. 7).
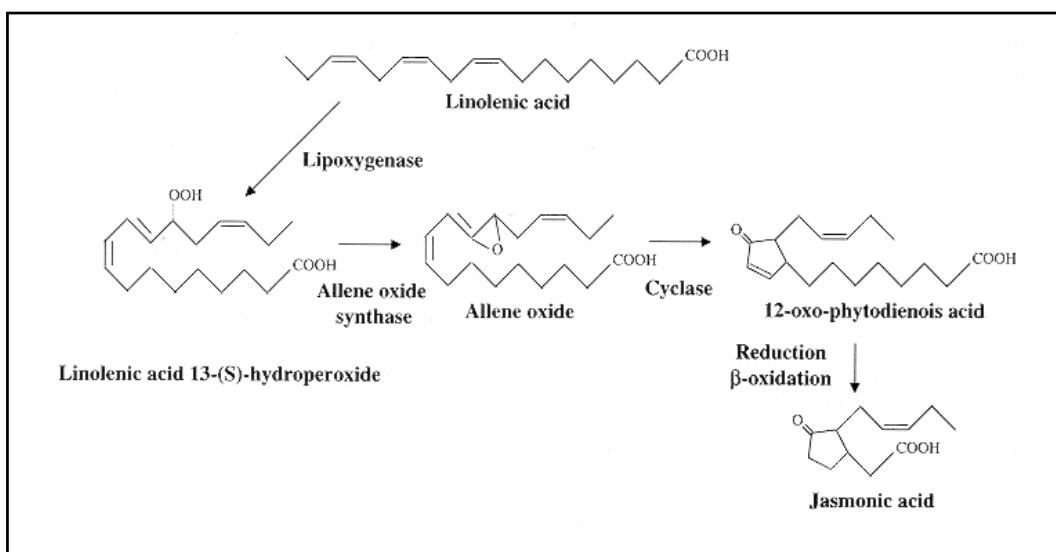


**Fig. 7.** Bioconversion of α-linolenic acid into jasmonic acid by the fungus *Botryodiplodia theobromae*.

The "grassy green notes" in damaged green tissue (e.g. cut grass) and in aromas of many fruits and vegetables are also formed via lipoxygenases, which degrade plant PUFA's (linolenic acid) and form hydroperoxides. These in turn are cleaved by hydroperoxide lyases to form the volatile aliphatic 6- to 9 carbon cis-3-hexenol (leaf alcohol) and trans-2-hexenal (leaf aldehyde). These enzymes have not been detected so far in bacteria or fungi; they are now obtained as by-products of the essential oil industry (Hatanaka, 1993). Alternatively, cloning of the plant genes, coding for lipoxygenase and hydroperoxides lyase in yeast cells, can allow for the formation of the green note flavour by fungal fermentation directly from added linolenic acid (Gallo *et al.*, 2001).

**Enzymatic flavour synthesis**

Despite the higher costs involved, microbial enzymes - rather than cells - can offer high stereo- and enantioselectivity towards substrate conversion (Schreier, 1997). Techniques such as enzyme immobilisation and eventually coenzyme regeneration might result in highly efficient and specific biocatalytic processes for flavour synthesis. A sophisticated example here is the conversion of 1-phenyl-2-propanone with $NADH + H^+$ dependent yeast alcohol dehydrogenase into (S)-1-phenyl-2-propanol; $NADH + H^+$ regeneration is obtained via coupling with a reaction, whereby formic acid is converted into gaseous $CO_2$ by formate dehydrogenase (Kragl *et al.*, 1996) (Fig. 8).
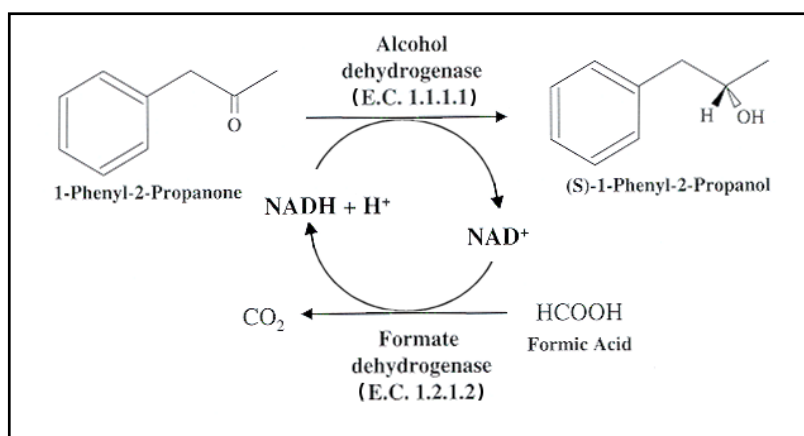


**Fig. 8.** Enzymatic synthesis of (S)-1-phenyl-2-propanol with cofactor regeneration via the coupled conversion of formic acid into $CO_2$.

**Fragrances via yeast fermentation**

2-Phenylethanol is an important flavour/fragrance component (threshold: 125 ppm) of certain fruit and beverage flavours, but also of rose fragrances. It can be produced chemically, or via extraction from roses; the naturally obtained product is extremely expensive. During conventional yeast fermentations (*Saccharomyces cerevisiae, Kluyveromyces marxianus*), low levels of 2-phenylethanol can be recovered from the fermentation gasses or broth using specific resins. To improve the process, *S. cerevisiae* mutants have been selected, which convert added L-phenylalanine via deamination, decarboxylation and subsequent reduction into 2-phenylethanol, without very little further metabolisation; high yields (> 2 g/l) are now obtained by solvent extraction of the fermentation broth (Lomascolo *et al.*, 2001) (Fig. 9).
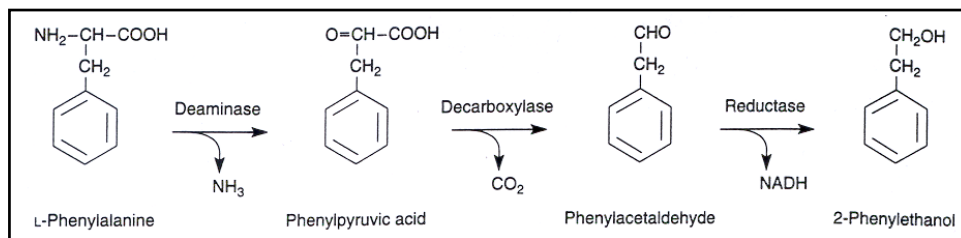
**Fig. 9.** Bioproduction of 2-phenylethanol from L-phenylalanine by *Saccharomyces cerevisiae* mutants.

Musk and civet components evaporate very slowly and fix other odours. In this respect, musks are important components of many fragrances; most are of a polycyclic aromatic nature and are produced via petro-chemical synthesis. Naturally occurring macrocyclic lactone musks are found in some plants (e.g. ambrette seed oil, galbanum), while keto-musks are produced by musk deer and civet cats, now very expensive and unethical sources (Cheetham, 1993).

Mutants of the yeast *Torulopsis bombicola* have been obtained which are able to convert palmitic acid into ω-hydroxypalmitic acid ester, which can then be cyclised into hexadecanolide lactone musk, with low yield however.

Another bioprocess has been described to produce the fragrance ingredient called Ambrox®, a terpene furan. It is one of the important components of ambergris, an excretion product of sperm whales. It is, just like musk, a fixative for other fragrances. A chemical process start from the terpene sclareol, extracted from the *Salvia sclarea* plant, which is converted via sclareolide into Ambrox®.

Also here, fungal and yeast strains (*Hyphozyma roseoniger*, *Cryptococcus* sp.) have been screened to be able to use sclareol as a sole carbon source and to accumulate sclareolide, which is then chemically converted via its diol into Ambrox® (Cheetham, 1993)

**Bottlenecks and opportunities**

Numerous fungal and yeast strains are capable of synthesizing potentially valuable flavour or fragrance compounds; however, yields are quite often disappointingly low, rarely above 100 mg/l, making these processes economically unattractive.

A better understanding of the fungal biochemistry and enzymes involved, metabolic regulation and genetic modification are primordial to improve yields, in addition to the application of novel fermentation technology and flavour recovery.

164

Screening should be intensified for new microbial strains, producing more efficiently - for example - vanillin from eugenol, or capable of forming β-ionon from β-carotene, green notes from linolenic acid or furanones from glucose.

Another challenge is the fact that many flavour compounds, or their added precursors (especially fatty acids, ricinoleic acid and fusel oil) are inhibitory or even toxic (at "higher" levels) to the producer strains. In this respect, slow continuous feeding of low precursor levels (fed batch fermentation), cell protection via immobilisation (Lee *et al.*, 1998) or *in situ* flavour extraction (via membranes and solvents (Hausler and Munch, 1997) are fermentation technologies, which could help to circumvent these limitations.

The cost of the raw materials, precursors and the formation and elimination of unwanted side products (even when present at traces) also add up to the economic viability of the bioflavour process, unless cheap side products such as fusel oils can be used.

Also the detection, identification and characterisation of novel microbial strains and their flavour compounds (mixture or top notes) needs to be further optimised. Due to their low threshold values (ppm to ppb) – though still easily detectable by the human nose - sophisticated sensitive, quantitative and continuous detection systems for such volatile substances (headspace analysis) have to be further developed.

It is clear from the above statements and examples that interdisciplinary cooperation between microbiologists, mycologists, biochemists, organic chemists and bioprocess engineers is a prerequisite to develop interesting findings in nature and in the laboratory into an industrial process for bioflavour production. Indeed, fungal biodiversity is enormous and remains largely untapped as to its potential to deliver a wide range of valuable metabolites.

## References

Berger, R.G. (1995). *Aroma Biotechnology*. Springer Verlag.

Bleumke, W. and Schrader, J. (2001). Integrated bioprocess for enhanced production of natural flavors and fragrances by *Ceratocystis moniliformis*. Biomolecular Engineering 17: 137-142.

Cheetham, P.S.J. (1993). The use of biotransformation for the production of flavours and fragrances. Trends in Biotechnology 11: 478-488.

Cheetham, P.S.J. (1997). Combining the technical push and the business pull for natural flavours. Advances in Biochemical Engineering and Biotechnology 55: 1-49.

Dahlen, T., Hauck, T., Wein, M. and Schwab, W. (2001). 2,5-dimethyl-4-hydroxy-3(2H)-furanone as a secondary metabolite from D-fructose-1,6-diphosphate metabolism by *Zygosacharomyces rouxii*. Journal of Bioscience and Bioengineering 91: 352-358.

Feron, G., Bonnarme, P. and Durand, A. (1996). Prospects in the microbial production of food flavours. Trends in Food Science and Technology 7: 285-293.

Gallo, A., Mita, G., Zasiura, C., Casey, R., Santino, A. and Zacheo, G., (2001). Recombinant lipoxygenases as biocatalysis for natural flavour production. Mededelungen Faculteit Landbouwwetenzhappen, Gent Universiteit 66/3a: 267-272.

Gatfield, I.L. (1997). Enzymatic and microbial generation of flavours. Agro-Food-Industry Hi-Tech 8: 35-38.

Hagedorn, S. and Kaphammer, B. (1994). Microbial biocatalysis in the generation of flavour and fragrance chemicals. Annual Review of Microbiology 48: 773-800.

Hatanaka, A. (1993). The biogeneration of green odour by green leaves. *Phytochem* 34: 1201-1218 .

Häusler, A. and Munch, T. (1997). Microbial production of natural flavours. ASM-News 63: 551-559.

Janssens, L., Depooter, H.L., Vandamme, E.J. and Schamp, N.M. (1987). Production of flavours by the yeast *Hansenula mrakii*. Mededelungen Faculteit Landbouwwetenzhappen Rijksuniversiteit, Gent 32: 1907-1913.

Janssens, L., Depooter, H.L., De Mey, L., Vandamme, E.J. and Schamp, N.M. (1989). Fusel oil as a precursor for the microbial production of fruity flavours. Mededelungen Faculteit Landbouwwetenzhappen Rijksuniversiteit, Gent 54(4a): 1387-1391.

Janssens, L., Depooter, H.L., Schamp, N.M. and Vandamme, E.J. (1992). Production of flavours by microorganisms. Process Biochemistry 27: 195-215.

Kragl, U., Kruse, W., Hummel, W. and Wandrey, C. (1996). Enzyme engineering aspects of biocatalysis: cofactor regeneration as example. Biotechnology and Bioengineering 52: 309-319.

Krings, U. and Berger, R.G. (1998). Biotechnological production of flavours and fragrances. Applied Microbial Biotechnology 49: 1-8.

Lee, S.L., Cheng, H.Y., Chen, W.C. and Chou, C.C. (1998). Production of γ-decalactone from ricinoleic acid by immobilized cells of *Sporidiobolus salmonicolor*. Process Biochemistry 33: 453-459.

Lomascolo, A., Lesage-Meesen, L., Haon, M., Navarro, D., Antona, C., Faulds, C. and Marcel, A. (2001). Evaluation of the potential of *Aspergillus niger* species for the bioconversion of L-phenylalanine into 2-phenylethanol. World Journal of Microbiolial Biotechnology 17: 99-102.

Palomares, M.R., Negrete, A., Miranda, L., Flores, C., Galindo, E. and Serrano-Carreon, L, (2001). The potential application of aqueous two-phase systems for in situ recovery of 6-pentyl-α-pyrone produced by *Trichoderma harzianum*. Enzyme Microbial Technology 28: 625-631.

Priefert, H., Rabenhorst, J. and Steinbüchel, A. (2001). Biotechnological production of vanillin. Applied Microbiology and Biotechnology 56: 296-314.

Schreier, P, (1997). Enzymes and Flavour Biotechnology. Advances in Biochemical Engineering Biotechnology 55: 51-72.

Stentelaire, C., Lesage-Meessen, L., Oddou, J., Bernard, O., Bastin, G., Collonna-Ceccaldi, B. and Asther, M. (2000). Design of a fungal bioprocess for vanillin production from vanillic acid at scalable level by *Pycnoporus cinnabarinus*. Journal of Bioscience and Bioengineering 89: 223.230.

Takahara, S., Fujiwara, K. and Mizutani, J. (1973). Neutral constituents of volatiles in cultured broth of *Sporobolomyces odorus*. Agricultural and Biological Chemistry 37: 2855-2861.

Vanhooren, P. and Vandamme, E.J. (1999). L-fucose : occurrence, biological role, chemical and microbial synthesis. Journal of Chemical Technology and Biotechnology 74: 479-497.