ISSN 1330-9862
(FTB-1648)

review

Production of Food Aroma Compounds: Microbial and Enzymatic Methodologies

*María Asunción Longo and María Angeles Sanromán**

Department of Chemical Engineering, University of Vigo,
Campus Universitario As Lagoas – Marcosende, E-36310 Vigo, Spain

Received: December 1, 2005
Accepted: March 10, 2006

Summary

Over the past few years, the increasing demand for natural products in the food industry has encouraged remarkable efforts towards the development of biotechnological processes for the production of flavour compounds. The present paper reviews the recent major achievements reported in this field, with a special emphasis on the potential lying in plant cell, microbial cultures and enzyme technology for the production of a wide range of flavours. The use of solid-state fermentation as a means to improve economical feasibility of these processes is also considered.

Key words: aroma compounds, biotransformation, microorganisms, enzymes, food, plant cell cultures

Introduction

Flavour is usually the result of the presence, within complex matrices, of many volatile and nonvolatile components possessing diverse chemical and physicochemical properties. Whereas the nonvolatile compounds contribute mainly to the taste, the volatile ones influence both taste and aroma. A vast array of compounds may be responsible for the aroma of the food products, such as alcohols, aldehydes, esters, dicarbonyls, short to medium-chain free fatty acids, methyl ketones, lactones, phenolic compounds and sulphur compounds (*1,2*).

Since early times, flavour compounds ranging from single to complex substances have been extracted from plant sources. Eventually, after elucidation of their structure, synthetic flavours were produced by chemical synthesis. Nowadays, flavours represent over a quarter of the world market for food additives and most of the flavouring compounds are produced *via* chemical synthesis or by extraction from natural materials. However, recent market surveys have shown that consumers prefer foodstuff that can be labelled as »natural«. Although flavours may be produced by chemical transformation of natural substances, the resulting products cannot legally be labelled as natural. Furthermore, chemical synthesis often results in environmentally unfriendly production processes and lacks substrate selectivity, which may cause the formation of undesirable racemic mixtures, thus reducing process efficiency and increasing downstream costs. On the other hand, the production of natural flavours by direct extraction from plants is also subject to various problems. These raw materials often contain low concentrations of the desired compounds, making the extraction expensive. Moreover, their use depends on factors difficult to control such as weather conditions and plant diseases. The disadvantages of both methods and the increasing interest in natural products have directed many investigations towards the search for other strategies to produce natural flavours.

An alternative route for flavour synthesis is based on microbial biosynthesis or bioconversion (*3–6*). The most popular approaches involve the use of microbial cultures or enzyme preparations, although plant cell cultures have also been reported as suitable production systems (Fig. 1). Microorganisms can synthesize flavours as secondary metabolites during fermentation on nutri-

*Corresponding author; Phone: ++34 986 812 383; Fax: ++34 986 812 380; E-mail: sanroman@uvigo.es; Website: webs.uvigo.es/bioprocesos/

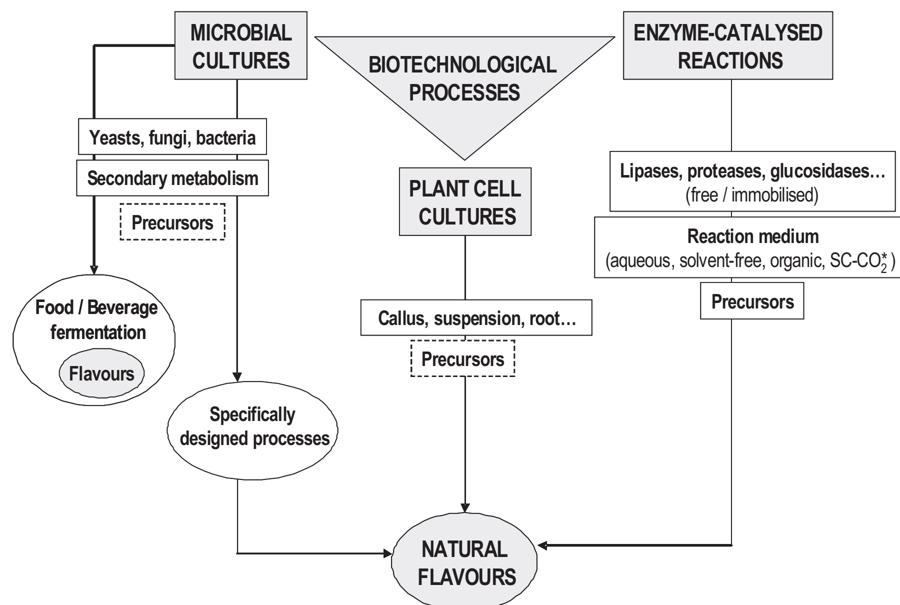

Fig. 1. Biotechnological processes for the production of flavour compounds
*SC-CO_2: supercritical carbon dioxide

ents such as sugars and aminoacids. This capability may be used in two different ways:

- *in situ* flavour generation, as an integral part of food or beverage production processes (*i.e.* cheese, yogurt, beer, wine) which determines the organoleptic characteristics of the final product,
- microbial cultures specifically designed to obtain aroma compounds that can be isolated and used later as additives in food manufacture. This strategy allows the obtained flavours to be labelled as natural.

In both cases, precursors or intermediates can be added to the culture medium in order to promote the biosynthesis of specific flavours. Also, the information obtained through the investigation of microbial metabolism in food fermentation processes could be utilized to develop suitable production systems for particular flavour additives. On the other hand, enzyme technology offers a very promising option for natural flavour biosynthesis. A number of enzymes (*i.e.* lipases, proteases, glucosidases) catalyse the production of aroma-related compounds from precursor molecules. The use of enzyme-catalysed reactions has the notable advantage of providing higher stereoselectivity than chemical routes. Besides, the products thus obtained may possess the legal status of natural substances.

Although a considerable amount of current research focuses on the production of food flavour and aroma compounds, at the moment only a few are obtained by biotechnological routes. The aim of this paper is to review the current state of the art on bioprocesses for the production of food flavours. The most relevant achievements related to plant cell and microbial flavour production, as well as enzyme-catalysed protocols are described. Finally, new approaches aiming to circumvent economic restrictions in biotechnological food aroma production are analysed.

Plant Cell Cultures

Plant cell cultures appear as a viable method to produce a wide range of flavours and aromas characteristic of their plant origin (Table 1, *7–16*). This approach is based on the unique biochemical and genetic capacity, and the totipotency of plant cells (*17–19*). Every cell of a plant culture contains the genetic information necessary to produce numerous chemical components that constitute natural flavour. Feeding intermediates of the biosynthetic pathway can enhance the production of flavour metabolites by precursor biotransformation. Some authors (*20,21*) summarised the advantages of plant cell culture technology over conventional agricultural production, pointing out the following aspects:

Table 1. Flavours from plant cell cultures

Products	Plant species	References
2,3-butanedione, (E,Z)-2,6-nonadienal and (E,Z)-2,6-nonadien-1-ol	*Agastache rugosa*	(*7*)
Apple aroma	*Malus silvestris*	(*8*)
Cinnamic acid	*Nicotiana tabacum*	
Caryophyllen	*Lindera strychnifolia*	
Basmati flavour	*Oryza sativa*	(*9*)
Cocoa flavour	*Theobromo cacao*	(*10*)
Flavanol	*Polygonum hydropiper*	(*11*)
Garlic	*Allium sativum*	(*12*)
Monoterpenes	*Perilla frutescens*	(*13*)
Onion	*Allium cepa*	(*14*)
Triterpenoid	*Glycyrrhiza glabra glandulifera*	(*15*)
Vanillin	*Vanilla planifolia*	(*16*)

- it is independent of geographical and seasonal variations, political interference and other environmental factors. It also offers a defined production system, which ensures a continuous product supply, as well as uniform quality and yield,
- it is possible to produce novel compounds that are not normally found in parent plants, either directly or through stereo- and regiospecific biotransformations of cheap precursors,
- high-productivity species can be selected,
- costs can be decreased and productivity increased by automatization of cell growth control and regulation of metabolic processes,
- efficient downstream recovery,
- rapid production.

Nevertheless, some problems need to be solved before plant cell cultures are extensively used for plant metabolite production. The technology for large scale suspension cultures should be further developed, since it differs from that commonly employed for microbial systems. Sensitivity to shear stress, relatively long growth cycles, low yields, progressive loss of biosynthetic activity and rare product secretion are some of the features of plant cell cultures that need special attention. However, some strategies have been developed to stimulate biosynthetic activities of cultured plant cells by optimization of culture conditions, selection of high-producing strains, precursor feeding and elicitation. Also, cell immobilisation techniques could help to prolong viability periods, maintain high cell density in the bioreactors and reduce shear stress, amongst other advantages. Besides, regulation of plant secondary metabolism at the biochemical and genetic levels could lead to improved production systems (*21,22*).

As for specific efforts related to flavour production by plant cell cultures, several researchers have investigated the synthesis of vanillin, a much sought-after flavour compound (*23*). Plant cell cultures of *Vanilla planifolia* have been initiated from various plant cells and tissues (*24*), and the convenience of using elicitors to induce vanillic acid synthesis assessed (*25*). Also, feeding of the precursor ferulic acid resulted in increase in vanillin accumulation (*26,27*). Furthermore, the production of vanillin from ferulic acid with vanilla aerial roots on charcoal as a product reservoir has been described (*28*). *Capsicum frutescens* root cultures have also been used for the bioconversion of ferulic acid to vanillin (*29*).

Some other works involve the production of monoterpenes (*i.e.* limonene, linalool, *etc.*) in callus tissues and cell suspensions of *Perilla frutescens* (*13*), and basmati rice volatile flavour components in callus cultures of *Oryza sativa* (*9*). In some cases, the flavour profiles obtained in plant cell cultures differ from those encountered in the parent plants. Such was the case in suspension cultures of *Agastache rugosa* Kuntze (Korean mint), which had a marked cucumber/wine-like aroma, and produced some interesting flavour-related alcohols (*i.e.* 2-phenylethanol) (*7*). This alteration of the original flavour profiles can be deliberately induced by the addition of precursors, as demonstrated in root cultures of *Allium cepa* L. (onion) (*14*).

Microbial Cultures

Microorganisms have historically played an integral role in the elaboration of the flavour components of many different foods. Products such as wine, vinegar, beer, fermented vegetables, milk, soya and meat have been preserved, modified and flavoured by means of microbial strains. As previously indicated, microbial cultures can be used to produce flavour compounds, either specifically for application as food additives or *in situ* as a part of food fermentation processes.

Aroma compounds can be classified in families based on their chemical structures, physicochemical or sensorial properties (Table 2). Alternative classifications could be established as a function of the chemical family of the precursor used for their production by bioconversion. Detailed information on the production of some commonly used food aroma compounds by microorganisms is presented below.

Diacetyl

Diacetyl is mainly related to butter flavour, and therefore extensively used in the imitation of butter and other dairy flavours, as well as whenever butter notes are desirable in food or beverages. This compound is produced by lactic acid bacteria and other microorganisms in several foods (*e.g. Lactococcus lactis, Lactobacillus* sp., *Streptococcus thermophilus, Leuconostoc mesenteroides*). The production of dairy flavour compounds, such as butyric acid, lactic acid and diacetyl in mixed cultures of lactic acid bacteria growing in starch-based media, has been reported (*30*). The studies done by Ibragimova *et al.* (*31*) showed that milk cultures of *Streptococcus lactis, S. cremoris* and *S. diacetilactis* produced high amounts of 2,3-butanedione and acetaldehyde in 24 h at 30 °C. Cultures with the best aroma contained 2–5 parts acetaldehyde to 1 part 2,3-butanedione.

A number of researchers have investigated the behaviour and/or metabolism of food processing microorganisms, and the enzymes involved in the production of diacetyl and related compounds. The formation of diacetyl by lactic acid bacteria through acetoin dehydrogenase-catalysed dehydrogenation of acetoin has been investigated in the dough products (*32*). Bassit *et al.* (*33*) studied the effect of temperature on diacetyl and acetoin production by a particular strain of *Lactococcus lactis*, with special reference to lactic dehydrogenase, acetolactate synthase, NADH oxidase and diacetyl reductase, the main enzymes involved in pyruvate metabolism. Medina de Figueroa *et al.* (*34*) investigated the effect of citrate in the repression of diacetyl/acetoin reductase, resulting in the accumulation of diacetyl and acetoin in batch cultures of *Lactobacillus rhamnosus*.

Genetic manipulation of the gene encoding enzymes involved in diacetyl metabolism, such as diacetyl-acetoin reductase from *Lactococcus lactis*, has been attempted to increase the diacetyl production capacity of lactic acid bacteria (*35*). Also, Carroll *et al.* (*36*) cloned and expressed in *E. coli* acetolactate synthase, a key enzyme for the production of the diacetyl precursor acetolactate, with the final objective of increasing diacetyl production in lactococcal strains.

Table 2. Classification of food aroma compounds based on their chemical structure

Alcohols	Aldehydes	Esters	Fatty acids	Ketones
1,2-butanediol	acetaldehyde	methyl acetate	acetate	acetophenone
2-butanol	decanal	ethyl acetate	butyrate	acetone
2-3-butanediol	heptanal	ethyl butyrate	caproate	2,3-butanedione
ethanol	(Z)-4-heptenal	ethyl hexanoate	decanoate	2,3-pentandione
2-ethylbutanol	hexanal	ethyl isobutanoate	isobutyrate	2-butanone
2-ethylhexanol	2-hexenal	ethyl octanoate	2-methylbutyric acid	3-hydroxy-2-butanone
2-heptanol	isohexanal	ethyl butanoate	3-methylbutyric acid	2-heptanone
hexanol	2-methylbutanal	isobutyl butanoate	octanoate	2-hexanone
isobutanol	3-methylbutanal	2-methyl-1-butyl acetate	phenylacetate	3-methyl-2-butanone
2-methylbutanol	2-methylpropanal	3-methyl-1-butyl acetate	propionate	4-methyl-2-pentanone
3-methylbutanol	nonanal	3-octyl acetate	valerate	2-nonanone
2-methylpropanol	(E,E)-2,4-nonadienal	pentyl acetate	**Lactones**	2-octanone
2-nonanol	(Z)-2-nonenal	phenethyl acetate	δ-decalactone	1-octen-3-one
(Z)-1,5-octadien-3-ol	(E)-2-nonenal	ethyl butyrate	γ-decalactone	2-pentanone
2-octanol	octanal	propyl butyrate	γ-butyrolactone	3-pentanone
1-octen-3-ol	butanal	2-hidroxyethyl	δ-dodecalactone	2-tridecanone
1-pentanol	pentanal	propionate	δ-octalactone	2-undecanone
phenylethanol	propanal	2-methyl-2-ethyl-3-	(Z)-6-dodecen-δ-lactone	
2-phenylethanol	propenal	-hydroxyhexyl propionate		
1-nonanol	thiophen-2-aldehyde	ethyl 2-methylbutanoate		
		ethyl 3-methylbutanoate		

Aromatic compounds	Pyrazines
vanillin	2,3-diethyl-5-methylpyrazine
benzaldehyde	2-ethyl-3,5-dimethylpyrazine
β-phenethyl alcohol	2-methoxy-3-isopropylpyrazine
trimethylbenzene	

In some cases, diacetyl can contribute to off-flavours (*i.e.* beer production) and strategies should be designed to avoid their formation. Kronlof and Linko (*37*) proposed the use of genetically modified brewer's yeast encoding α-acetolactate decarboxylase in immobilised yeast bioreactors for the main fermentation of beer, promoting the direct conversion of α-acetolactate to acetoin without the formation of diacetyl. Also, Sandine *et al.* (*38*) assayed the addition of a crude diacetyl reductase from *Aerobacter aerogenes* as a means to remove diacetyl and 2,3-pentadione from beer by conversion to flavourless acetoin.

Lactones

Lactones are cyclic esters of primarily γ- and δ-hydroxy acids, and they are ubiquitously found in food, contributing to taste and flavour nuances such as fruity, coconut-like, buttery, creamy, sweet or nutty. The possibility of producing a lactone using a biotechnological route was discovered in the 1960s by the group of Okui (*39,40*) during the investigation of hydroxyacid catabolism by several organisms. Dimick *et al.* (*41*) stated in their review that raw milk does not contain free lactones, which only appear after heating. The milky, buttery and coconut-like flavour notes provided by these compounds are generally considered as desirable in dairy products. However, the presence of lactones may contribute to the stale flavour of heated milk, although to a lesser extent than ketones. The compound 6-pentyl-2-pyrone provides a coconut aroma, highly desired by flavourists. It was found by Collin and Halim (*42*) to be the major volatile constituent in cultures of the fungus *Trichoderma viride*. Other fungi such as *Tyromyces sambuceus* and *Cladosporium suaveolens* efficiently generate the coconut-flavoured lactones γ-decalactone and δ-dodecalactone from ricinoleic acid and linoleic acid, respectively (*43,44*).

Yeasts such as *Candida tropicalis* or *Yarrowia lipolytica* degraded ricinoleic acid to C16, C14 and C12 acids and, interestingly, accumulated δ-decalactone, which exhibits fruity and oily notes important in the formulation of peach, apricot or strawberry aromas. However, the yields of this biotransformation are commonly poor, and they rarely reach concentrations over 4 to 5 g/L in the fermentation broth (*45*). Wache *et al.* (*46*) investigated the enzymes involved in γ-decalactone production by *Yarrowia lipolytica*, and encountered the reasons for low yields.

Esters

Esters are commonly used flavouring agents, very appreciated for the fruity aromas they provide. They are employed in fruit-flavoured products (*i.e.* beverages, candies, jellies, and jams), baked goods, wines, and dairy products (*i.e.* cultured butter, sour cream, yogurt, and cheese). Acetate esters, such as ethyl acetate, hexyl acetate, isoamyl acetate and 2-phenylethyl acetate are recognised as important flavour compounds in wine and other grape-derived alcoholic beverages. Rojas *et al.* (*47*) studied several so-called non-*Saccharomyces* wine yeasts as the producers of acetate ester. Among them, the yeasts *Hanseniaspora guilliermondii* and *Pichia anomala* were found to be potent 2-phenylethyl acetate and isoamyl acetate producers, respectively.

In cheese production, ethyl or methyl esters of short--chain fatty acids generally bring about fruity flavours, while thioesters derived from thiols are associated with cabbage or sulphur aromas (*48*). The capacity of lactic acid bacteria to synthesize both ethyl esters and thioesters has been reported. The role of a unique esterase from *Lactococcus lactis* in the formation of these aroma compounds has been investigated, and ascertained as at least partially responsible for the esterification reactions leading to the production of aroma ester compounds. This was undertaken by using an esterase negative mutant of *L. lactis* (*49*).

Pyrazines

Pyrazines are heterocyclic, nitrogen-containing compounds which possess a nutty and roasted flavour. They are normally formed during conventional cooking/roasting of food through the Maillard reaction (*50*). Nowadays, the use of cooking processes that do not favour pyrazine formation (*i.e.* microwave cooking) has caused the need to supply natural pyrazines with a roasty flavour as food additives. A few microorganisms are also able to synthesize pyrazines. For instance, bacteria such as *Corynebacterium glutamicum* produce important quantities of tetramethylpyrazine from amino acids (*51*).

Terpenes

Terpenes are widespread in nature, mainly in plants as constituents of essential oils. They are composed of isoprene units, and can be cyclic, open-chained, saturated, unsaturated, oxidized, *etc*. The biotransformation of these compounds is potentially of considerable interest for application in the food flavour industry. Among the terpenes, linalool, nerol, geraniol and citronellol are the most flavour-active due to their low sensory threshold.

Most of the terpenes obtained in microbial cultures are produced by fungi that belong to the ascomycetes and basidiomycetes species. Schindeler and Bruns (*52*) have demonstrated that terpene yields in *Ceratocystis variospora* cultures could be improved when toxic end products were removed using ion exchange resins. The fungus *Ceratocystis moniliformis* produces several aroma products such as ethyl acetate, propyl acetate, isobutyl acetate, isoamyl acetate, citronellol and geraniol. In order to avoid the inhibitory effects detected in these cultures, it is necessary to decrease product concentrations in the bioreactor. Bluemke and Schrader (*53*) developed an integrated bioprocess to enhance the production of natural flavours by *C. moniliformis*. The total yield of aroma compounds produced in the integrated bioprocess, with *in situ* product removal using pervaporation, is higher than in conventional batch cultivation. In addition, permeates obtained from pervaporation consist of highly enriched mixtures of flavours and fragrances. On the other hand, microbial transformation of terpenes has received considerable attention. Many microorganisms are able to break down terpenes or to carry out specific conversions, creating products with an added value. Dhavlikar and Albroscheit (*54*) demonstrated that the inexpensive sesquiterpene valencene can be converted by some bacteria to the important aroma compound nootkatone.

Recently, significant research effort has focused on the enzymes related to terpene biosynthesis. The nucleic acid sequence of a monoterpene synthase from sweet basil, a key enzyme for the production of geraniol, has been determined in order to allow the production of recombinant geraniol synthase (*55*). Also, a geraniol synthase from the evergreen camphor tree *Cinnamomum tenuipilum* was cloned and expressed in *E. coli* (*56*). Functional genomics has also been applied to identify the genes for monoterpene synthases from *Vitis vinifera* grapes in order to characterize the enzymes by expression in *E. coli* and subsequent analysis (*57*).

Alcohols

In alcoholic fermentations, apart from ethanol, yeast produces long-chain and complex alcohols. These compounds and their derived esters have interesting organoleptic properties. Some authors have proposed strategies for promoting this kind of flavour compounds during alcoholic beverage production. Mallouchos *et al.* (*58*) utilized *Saccharomyces cerevisiae* immobilised on delignified cellulosic material and gluten pellets. The former produced higher amounts of esters, whereas the latter gave higher amounts of alcohols. Kana *et al.* (*59*) evaluated yeast immobilisation on γ-alumina and kissiris, and found in the former case an increase in the concentration of amyl alcohols, total volatiles, and ethyl acetate, which led to a fine aroma.

One of the most relevant aroma-related alcohols is 2-phenylethanol, which possesses a rose-like smell. It is still predominantly synthesized by petrochemical routes from toluene, benzene, styrene, or methylphenylacetate (*60*), while the natural 2-phenylethanol is mainly extracted from rose petals through a high-cost process (*61*). Different yeast strains such as *Hansenula anomala*, *Kluyveromyces marxianus* or *Saccharomyces cerevisiae* have shown a high potential for industrial production of aroma compounds, such as 2-phenylethanol, which is derived from 2-phenylalanine by bioconversion (*61,62*). Stark *et al.* (*62, 63*) reported that the presence of ethanol and 2-phenylethanol in the medium resulted in a synergistic inhibition, which reduced the tolerance of *Saccharomyces cerevisiae* to 2-phenylethanol and thus its final concentration. As a result, the feed rate had to be reduced in fed-batch cultures to avoid ethanol production. Thus, a maximal 2-phenylethanol concentration of 2.35 g/L could be attained in batch cultures, whereas 3.8 g/L were obtained in a fed-batch culture with the limitation of ethanol pro-

duction (*62*). To enhance the productivity of the bioconversion of 2-phenylalanine by *Saccharomyces cerevisiae*, a novel *in situ* product recovery strategy was proposed by Serp *et al.* (*64*). An organic solvent (dibutyl sebacate) was entrapped within a polyethylene matrix, in order to form a highly absorbent, chemically and mechanically stable composite resin. The use of this technique increased twofold the volumetric productivity of 2-phenylethanol and significantly facilitated downstream processing. Fabre *et al.* (*65*) screened 21 yeast strains for 2-phenylethanol production. Amongst the different 2-phenylethanol producers, *Kluyveromyces marxianus* was outstanding, which makes this strain a promising candidate to be applied in an industrial process. Moreover, *K. marxianus* has several advantages such as (*66*):

- it shows optimal production characteristics (*65*),
- 2-phenylethanol production depends on the medium and temperature used (*67*),
- *K. marxianus* is Crabtree-negative, which is an advantage for scale production processes, because the production of toxic by-products (*i.e.* ethanol) under aerobic conditions can be avoided (*68*).

Vanillin

Vanillin (4-hydroxy-3-methoxybenzaldehyde) is a universally appreciated flavour chemical that occurs in *Vanilla planifolia* beans. It is widely used in foods, beverages, perfumes, pharmaceuticals, and in various medical industries (*69*).

Chemically synthesized vanillin accounts nowadays for more than 99 % of the total market share (*70*), but there is an increasing demand for natural vanillin. Direct extraction from vanilla beans is expensive and limited by plant supply, which makes this compound a promising target for biotechnological flavour production. Vanillin is an intermediate in the microbial degradation of several substrates, such as ferulic acid, phenolic stilbenes, lignin, eugenol and isoeugenol. The conversion of natural eugenol and isoeugenol from essential oils into vanillin has been investigated using microbial and enzymatic biotransformations (*71–75*). Strains including *Pseudomonas putida*, *Aspergillus niger*, *Corynebacterium glutamicum*, *Corynebacterium* sp., *Arthrobacter globiformis* and *Serratia marcescens* (*69*) can also convert eugenol or isoeugenol to vanillin.

A two-step bioconversion process using filamentous fungi was developed by Lesage-Meessen *et al.* (*76,77*) to transform ferulic acid into vanillin. First, *Aspergillus niger* transformed ferulic acid to vanillic acid, and then vanillic acid was reduced to vanillin by *Pycnoporus cinnabarinus*. Bonnin *et al.* (*78*) showed that the yield of vanillin may be significantly increased by adding cellobiose to *P. cinnabarinus* culture medium, due to the decrease in oxidative decarboxylation of vanillic acid.

The importance of ferulic acid as precursor of vanillin has brought about a number of efforts in the investigation of its production. Feruloyl esterase has been identified as the key enzyme in the biosynthesis of ferulic acid, and some researchers have studied the production of this enzyme in microbial cultures of several fungi grown on different pretreated cereal brans, such as wheat, maize, rice bran and sugar cane bagasse (*79*). The metabolism of ferulic acid in some microorganisms has also been investigated (*80,81*).

Benzaldehyde

It is the second most important molecule after vanillin for its use in cherry and other natural fruit flavours. The world consumption of benzaldehyde amounts to approximately 7000 tonnes per year (*82*). Natural benzaldehyde is generally extracted from fruit kernels such as apricots, leading to the undesirable formation of the toxic hydrocyanic acid. Nowadays, the fermentation of natural substrates is an alternative route to the production of benzaldehyde without harmful by-products. However, benzaldehyde is toxic towards microbial metabolism and its accumulation in the culture medium may strongly inhibit cell growth (*83*). For this reason, only a few microorganisms have been reported as benzaldehyde producers. Amongst them, the bacterium *Pseudomonas putida* (*84–86*) and the white rot fungi *Trametes suaveolens*, *Polyporus tuberaster*, *Bjerkandera adusta* and *Phanerochaete chrysosporium* (*87–90*) are mentioned as biocatalysts in the biosynthesis of benzaldehyde from phenylalanine. Park and Jung (*91*) proposed the use of calcium alginate-encapsulated whole-cell enzymes from *P. putida* for the production of benzaldehyde from benzoylformate. This allowed the accumulation of benzaldehyde in the capsule core, minimising its subsequent transformation to benzyl alcohol by the action of alcohol dehydrogenase, and thus providing continuous production of benzaldehyde until reactant exhaustion.

Methyl ketone

The methyl ketones, 2-heptanone, 2-nonanone, and 2-undecanone, are the largest contributors to stale flavour in UHT milk (*92*). Moio *et al.* (*93*) similarly report that 2-heptanone and 2-nonanone are the most powerful odorants in UHT milk. These methyl ketones are aromas employed in a wide range of flavouring applications, especially those related to blue cheese and fruit flavours (*94*). There is not much information on microbial production of these compounds, although Janssens *et al.* (*3*) mention in their review the methyl ketone-producing ability of *Agaricus bisporus*, *Aspergillus niger*, *Penicillium roqueforti* and *Trichoderma viride* TS.

Enzyme-Catalysed Reactions

Chemical synthesis of food aromas is nowadays under question, due to drawbacks such as poor reaction selectivity leading to undesirable side reactions, low yields, pollution, high manufacturing costs, and impossibility of labelling the resulting products as natural. Besides, many compounds used for flavouring exist as optical isomers with different flavour characteristics, and resolution of racemic mixtures is usually difficult to achieve chemically. On the other hand, the use of enzyme-catalysed reactions circumvents many of these problems, due to the substrate specificity, regio- and enantioselectivity of these biocatalysts, which can be utilized at mild reaction conditions. By choosing the suitable enzymes, enantiomerically pure flavour compounds can be obtained in one step, thus increasing process efficiency

and lowering downstream costs. Moreover, flavours obtained through biocatalysis can be considered as natural products.

A number of enzymes may directly produce flavour molecules by hydrolysis of larger progenitors. Also, recent developments on biocatalysis in unconventional media have made possible the utilisation of hydrolytic enzymes to specifically catalyse the synthesis of a number of valuable compounds (*95*). This strategy can be applied for the production of food aromas, as is the case of ester synthesis by lipases in low water-content media. The most commonly used enzyme applications related to the production of aroma compounds are described below in some detail.

Lipolytic enzymes

One of the most promising applications of enzyme technology in the food aroma field is the use of reversed lipolysis in low water-content systems (*95*) in order to carry out esterification or transesterification reactions for the production of esters from inexpensive raw materials (*i.e.* fatty acids and alcohols). A number of lipases have been tested for their ability to promote ester synthesis in low-water content media, such as those from *Candida cylindracea*, *Pseudomonas fluorescens*, *Mucor miehei*, *Aspergillus* sp., *Rhizopus arrhizus* and *Candida rugosa*, amongst others. The influence of variables such as reagent or enzyme characteristics and concentrations, temperature, water content or solvent properties have been thoroughly studied in many cases. Also, the improvement of enzyme substrate specificity and the use of new solvents are under investigation.

Lipases are usually highly specific, which makes esterification between carboxylic acids and alcohols dependent on alcohol and/or acid chain length. Kumar *et al.* (*96*) studied the esterification of fatty acids of different chain lengths with isoamyl alcohol with three different commercial lipases. *Candida antarctica* lipase fraction B showed substrate specificity involving both acids (short-chain fatty acids having linear and branched chain structures, as well as unsaturated fatty acids), and alcohols (*n*-butyl, isopentyl, 2-phenylethyl and geraniol) when the synthesis of esters in *n*-hexane was considered (*97*). The conversion rate for isoamyl acetate, propionate, butyrate and octanoate synthesis with crude hog pancreas lipase under solvent-free conditions and in supercritical carbon dioxide increased with increasing carbon chain length for even-carbon acids, and higher yields were found for isoamyl propionate (*98*). Macedo *et al.* (*99*) found that the size of the aliphatic chain from the acyl donor was important for conversion rate, when production of citronellyl esters by direct esterification and transesterification catalysed by a novel microbial lipase from a locally isolated *Rhizopus* sp. strain was assessed.

As indicated above, enantiomeric selectivity of the enzymes can be an important feature for the synthesis of food aromas. For instance, only the (S)-form of 2-methylbutanoic acid methyl ester (main flavour component of apple or strawberry) has the characteristic fruit flavour. Kwon *et al.* (*100*) investigated the ability of a number of lipases to catalyse enantiomeric selective synthesis of (S)-2-methylbutanoic acid methyl ester, and found good results for lipases from *Rhizomucor miehei*, *Aspergillus niger* and *Aspergillus javanicus*. The reaction conditions (*i.e.* temperature and lyophilizing pH) were optimized in isooctane, and the kinetic parameters determined. Also, *Candida rugosa* lipase was selected to catalyse the esterification of L-menthol with long-chain unsaturated fatty acids in a solvent-free system in order to moderate its strong flavour. The enzyme acted strongly on L-menthol and very weakly on D-menthol (*101*). On the other hand, some lipases show only moderate enantioselectivity, as is the case of *Candida antarctica* lipase fraction B towards chiral short-chain carboxylic acids (*97*).

Kinetics of lipase-catalysed esterification and transesterification reactions has been investigated in detail by some authors. The synthesis of geranyl acetate by transesterification catalysed by immobilised *Mucor miehei* lipase in supercritical carbon dioxide and *n*-hexane appeared to follow a ping pong bi bi model with inhibition by excess of geraniol (*102,103*). A ping pong bi bi model with competitive substrate inhibition and dead-end inhibition by acetic acid was also reported for geranyl acetate formation by *Candida cylindracea* lipase-catalysed esterification in organic solvent (*104,105*).

Ester synthesis reactions are carried out in low-water content media. The most commonly used systems are organic solvents such as *n*-hexane (*97,99,102,103,106–110*), *n*-heptane (*111*), cyclohexane (*112*) or isooctane (*100, 105*). The choice of solvent has been found to be significant, and a relationship was suggested between solvent polarity, expressed in terms of log P, and reaction conversion. Highly hydrophobic solvents are preferred because they do not penetrate the water layer surrounding the enzyme surface, which favours maintenance of its active stereoconfiguration (*104,111*). However, although high reaction yields can be obtained in the presence of organic solvents, mass transfer limitations often result in relatively low reaction rates.

An important aspect to consider when esterification reactions are carried out in organic solvents is the fact that water is produced during the reaction and its accumulation could cause a progressive decrease in yields. Although small amounts of water are required to maintain the active conformation of the enzymes, low water activities are necessary to promote ester synthesis reactions. Some strategies have been proposed to remove excess water from the reaction media, such as the application of salt hydrates (*113*), circulation of the reaction mixture through a packed column filled with water-adsorbing materials (*114*), pervaporation (*115*), sparging of dry inert gas (*116*) or continuous water removal by hetero-azeotropic distillation (*117*).

Enzymatic synthesis of esters can also be carried out in solvent-free systems, in which the reaction medium involves a reactant itself (*i.e.* an alcohol) as a solvent. The avoidance of the organic solvent is of great interest for food applications, since the costs and toxicity problems associated with its recovery could be eliminated. Some works illustrate the feasibility of solvent-free systems for lipase-catalysed ester synthesis reactions, and high yields have been reported (*96,98,99,101,118–121*). Nevertheless, mass transfer limitations and the subsequent low reaction rates may also occur in this case, due to the characteristic heterogeneity of the reactions (li-

pases are insoluble in organic solvents, including alcohols).

Supercritical fluids are defined as fluids above their critical temperature and pressure, having liquid-like densities and gas-like diffusivities. Therefore, they appear as suitable solvents for enzymatic reactions with mass-transfer limitations, allowing increased conversions for very low enzyme loadings and substrate concentrations. Also, the solubilities of reactants and products are dependent on pressure and temperature of the system, which would help to integrate reaction and downstream processing into a single step. Supercritical carbon dioxide (SC-CO_2) presents a number of advantages, such as low cost, inflammability, nontoxicity, near-ambient critical temperature (31.1 °C) and moderate critical pressure (73.8 bar). In view of all this, a number of researchers have investigated the efficiency of several lipases to catalyse esterification and/or transesterification reactions in SC-CO_2, and in some cases the results were successfully compared to those obtained when the reaction was carried out in the presence of organic solvents or in solvent-free systems (*96,98,102,103,122*). Anyhow, the specific equipment required to operate with supercritical fluids still hinders extensive commercial application of this approach.

Also, some authors have focused on the location of enzymes that could be able to synthesize commercially interesting esters in aqueous media, thus avoiding the problems associated with organic, solvent-free or supercritical systems. Chang *et al.* (*123*) confirmed the ability of wild-type and mutant lipases from *Staphylococcus epidermidis* to catalyse the synthesis of various flavour esters in aqueous media, and studied the optimum reaction conditions. The enzymes were found to be strongly inactivated by *n*-hexane, and showed promising specificity for the synthesis of medium-chain esters, geranyl esters and unsaturated esters, although further genetic engineering could be considered to provide activity towards the synthesis of short-chain esters.

One of the main drawbacks for industrial application of enzyme-catalysed ester synthesis is the relatively high cost of the biocatalyst. Therefore, enzyme immobilisation strategies have been considered, which would allow recovery and reuse of the biocatalyst, and in some cases would help to increase enzyme thermostability and tolerance to organic solvents. Lipases from several sources were immobilised on supports such as Celite 545 (*122*), silica gel 60 (*123*), nylon (*106*) or macroporous anionic resins (*102,103*), and their efficiency in the catalysis of esterification and/or transesterification reactions in aqueous, organic, solvent-free or supercritical media was assessed. The characteristics of the immobilisation support (*i.e.* hydrophobicity) were reported to have an influence on ester synthesis reaction rates in organic solvents (*112*). Some novel immobilisation techniques have been proposed, such as magnetic polysiloxane-polyvinyl alcohol particles (*124*), and lipase-containing organic-inorganic hybrid sol-gel matrices within the pores of polyvinyl formal resin plates (*125*). The former showed high activity recovery during the covalent immobilisation process, and reaction conditions, including substrate selectivity towards organic acids and alcohols, were studied using heptane as solvent. With respect to the latter, optimum formulation of the sol-gel solution for enzyme activity was investigated, and excellent thermal and operational stability of the immobilised biocatalyst were found. The esterification reaction between geraniol and acetic acid in anhydrous hexane was assayed. Lipase coating with surfactants (*i.e.* Span 85) as a means to facilitate recovery and repeated use of the enzyme, due to the decreased solubility in organic solvents, was also assessed for the catalysis of geranyl acetate formation by esterification (*104,105*).

The use of enzymes produced by thermotolerant or thermophilic microorganisms could also help to diminish the costs associated with the biocatalysts in this kind of process, since the enzymes provided by these strains usually have extremely high thermostability and tolerance to organic solvents. Alvarez-Macarie and Baratti (*126*) assessed the ability of a novel thermostable esterase from the thermotolerant strain *Bacillus licheniformis*, cloned in *E. coli*, to catalyse the formation of short-chain flavour esters (ethyl caproate) in *n*-heptane. The effect on reaction yield of enzyme concentration, added water, fatty acid chain length specificity (higher for mid-chain length fatty acids), solvent, substrate concentration and temperature was investigated. The operational stability of the enzyme (*i.e.* repeated use) was assessed. Also, the ability of some *Thermus* strains to produce thermophilic lipolytic enzymes has been reported (*127–129*). These enzymes showed good tolerance to high temperatures and organic solvents, which indicates promising characteristics for their application in ester synthesis.

Proteases

Oligopeptides are receiving increasing attention due to their organoleptic properties and biological activity. Relatively short oligopeptides play an important role in the sensory appreciation of food, and different sequences can be used to recreate the four basic taste sensations (sweet, bitter, sour and salty) (*130,131*).

Peptides can be obtained by protease-mediated hydrolysis of proteins. The influence of the original protein source and the protease utilized for its hydrolysis on the range of obtained peptides and their taste characteristics has been studied (*131*). Sawhill (*132*) reported the production of a nonallergenic peptide product by enzymatic hydrolysis of whey protein concentrates with a fungal protease in a two-stage process. The product did not have the bitter taste typically associated with peptides, and its potential application for milk replacement products or other food applications was proposed. However, hydrolysis of proteins often results in the formation of hydrophobic bitter peptides, which limits the use of protein hydrolysates in food products. This bitterness can be reduced by masking, extraction of bitter peptides or further hydrolysis with exopeptidases (*133*).

Protease-catalysed hydrolysis can also influence the occurrence of other flavour-related compounds. Baek and Cadwallader (*134*) investigated protease treatment of crayfish-processing byproducts to obtain flavour concentrates, and found that the enzymatic treatment allowed an increase in the concentration of benzaldehyde and pyrazines. Aaslyng *et al.* (*135*) studied protease-mediated catalysis as an alternative for the production of

hydrolyzed vegetable protein, a savoury flavouring product traditionally obtained by heating a protein source at acidic pH. Enzymatic treatment favoured pyrazyne formation through Maillard reactions, as well as alcohols.

Peptides can be synthesized by recombinant DNA technology, chemical synthesis, and enzymatic synthesis, depending on the desired size (large, intermediate and short sequences, respectively). The use of enzymes circumvents some of the problems associated with chemical synthesis, since enzyme regio- and stereospecificity dispense the requirements for intermediate protection/deprotection steps and avoid racemization, while allowing mild reaction conditions. Therefore, the enzymatic method is particularly interesting for the synthesis of peptides to be used as food additives. The majority of commercially available proteases (metallo-, endo-, exo-, serine and aspartate) may be used, although the serine and cystein endopetidases have been the most widely employed. Application of free, suspended, immobilised and chemically modified enzymes has been proposed (*136*).

The use of an aqueous medium for the enzymatic synthesis reaction brings about some problems, such as the cleavage of preformed peptide bonds or the low solubility of commonly used protected amino acid derivatives. Organic cosolvents can be added to diminish the occurrence of hydrolytic side reactions and increase reagent solubility. Also, precipitation or extraction of the product could help to shift the equilibrium towards synthesis. The utilization of protease-catalysed peptide condensation reactions to synthesize valuable peptides or modify the structure of bitter peptides, thus reducing their bitterness, has been studied by a number of researchers. A dipeptide condensation reaction catalysed by papain and several commercial neutral and alkaline proteases was investigated by Stevenson *et al.* (*133*). Both water and aqueous ethanol were considered as reaction media, and the equilibrium towards peptide condensation was found to be considerably favoured by product precipitation. Also, the ability of the tetrapeptide Asp-Asp-Asp-Asp, isolated from beer yeast seasoning, to significantly mask bitter taste was ascertained, and the possibility of synthesizing it by oligomerization of L-aspartic acid α,β-di-propyl ester hydrochloride with α-chymotrypsin was demonstrated (*137*). Jorba *et al.* (*138*) proposed the use of semiliquid eutectic mixtures of substrates instead of aqueous or organic solutions in order to circumvent low productivity and limited choice of suitable solvents, often found as drawbacks for this methodology.

The use of enzyme preparations including proteases, as well as peptidases, lipases and esterases, to produce enzyme-modified flavour-enhanced cheese has been recommended by some authors. The procedure consists of incubating cheese or curd with enzymes in a slurry system under controlled conditions until the desired flavour is achieved, and flavour enhancements up to 30-fold compared to natural cheese can be reached. Apart from enzyme solutions, specific starter cultures providing the adequate enzyme pool can also be used (*139*). Some authors have stressed the potential antihypertensive properties of some peptides found in enzyme-modified cheese (*140*).

Glucosidases

Glucosidases can be used to enhance the aroma of some wines by freeing glycosidically bound volatile terpenes and flavour precursors. Terpenols in grapes are mostly found in glycosidically bound forms which are odourless. The addition of exogenous enzymes during or after the fermentation has been found to be the most effective way to improve the hydrolysis of the aroma precursor compounds and achieve an increase in wine flavour (*141*). This phenomenon is now exploited by enzyme companies who specifically produce β-glucosidase enzymes for the enhancement of the aroma of certain wines. β-glucosidases have been isolated from numerous sources including *Vitis, Saccharomyces, Oenococcus, Aspergillus* or *Candida*.

Glucosidases can also be used to synthesize glycosides as »slow release« aroma compounds. Volatile flavours evaporate during storage leading to decrease in the right concentration at the moment of consumption, but if they are present in a bound, non-volatile form, they can be liberated upon heating, thus optimizing organoleptic characteristics of the consumed product. Glucosides are adequate derivatives as »slow release« flavours, due to their very low vapour pressures and the possibility of obtaining them as natural compounds. Enzymatic synthesis of geranyl glucoside has been investigated, using β-glucosidase in low water activity media. Since the yield was rather low, a system for continuous separation of the product was devised, composed of a spray two-phase column reactor, a hydrophobic microfiltration module and an alumina adsorption column as the main elements (*142,143*).

Other enzymes

Some other enzymes have been mentioned for their potential applicability in the production of flavour compounds. However, further research is needed before considering their commercial application. De Temiño *et al.* (*144*) investigated the use of an immobilised alcohol dehydrogenase from *Lactobacillus kefir* to synthesize (R)-phenylethanol from acetophenone in an organic solvent (hexane). The enzyme and its cofactor were entrapped in polyvinyl alcohol gel beads in order to enhance their stability in organic solvents and enable both cofactor diffusion and *in situ* regeneration. Also, hydroxylation of sesquiterpenes by (+)-germacrene A hydroxylase, an enzyme of the cytochrome P450 monooxygenase type, was undertaken, and some interesting results were obtained involving the production of nootkatone, a grapefruit flavour compound, from (+)-valencene (*145*).

Enzymatic synthesis of vanillin from vanillylamine using amine oxidase from *Aspergillus niger* has been reported, and a continuous production process using immobilised enzyme assessed (*146*). Vanillylamine can be isolated from capsaicin, a natural ingredient of peppers and capsicums. Nandakumar *et al.* (*147*) stressed the interest of microbial glutaminases as flavour enhancers in the food industry, due to their involvement in the synthesis of L-glutamic acid, the main compound responsible for the unique flavour of fermented soy sauce.

Solid-State Fermentation

Numerous microorganisms are capable of synthesizing potentially valuable flavour compounds and enzymes used in flavour manufacturing. However, yields are often disappointingly low, which hampers extensive industrial application. In the last decades there has been an increasing trend towards the utilisation of the solid-state fermentation (SSF) technique to produce several bulk chemicals and enzymes. SSF has been known from ancient times (approximately 2600 BC), and typical examples of this technique are traditional fermentations such as Japanese »koji«, Indonesian »tempeh« and French »blue cheese«.

SSF is defined as any fermentation process performed on a nonsoluble material that acts both as physical support and source of nutrients in absence of free flowing liquid (*148*). This technique reproduces natural microbiological processes like composting and ensiling. The low moisture content means that fermentation can only be carried out by a limited number of microorganisms, mainly yeasts and fungi, although some bacteria have also been used (*149*). In recent years, SSF has received more and more interest from researchers, since several studies on enzymes (*150*), flavours (*151*), colourants (*152*) and other substances of interest to the food industry have shown that SSF may lead to higher yields or better product characteristics than submerged fermentation (SmF). In addition, costs are much lower due to the efficient utilisation and value-addition of wastes (*153*). The main drawback of this type of cultivation concerns the scaling-up of the process, largely due to heat transfer and culture homogeneity problems (*154*). However, research attention has been directed towards the development of designs such as mixed solid-state bioreactor (*155*), rotating drum bioreactor (*156*) and immersion bioreactor (*157*), which overcome these difficulties.

Food and agricultural wastes are produced in huge amounts, and since they are rich in carbohydrates and other nutrients, they can serve as a substrate for the production of bulk chemicals and enzymes using SSF technique. The nature of the solid substrate employed is a very important feature. However, the solid substrate not only supplies the nutrients to the culture but also serves as an anchorage site for the microbial cells, and other factors such as particle size and moisture level/water activity are critical (*158–161*). SSF could be potentially useful for the production of flavour compounds (*162, 163*). Feron *et al.* (*151*) reviewed the prospects of microbial production of food flavours and the recommended SSF processes for their manufacture.

Several researchers have studied SSF production of aroma compounds by several microorganisms (Table 3, *164–195*), such as *Neurospora* sp. (*167*), *Zygosaccharomyces rouxii* (*168*) and *Aspergillus* sp. (*172*), using pre-gelatinised rice, miso and cellulose fibres, respectively. Bramorski *et al.* (*196*) compared fruity aroma production by *Ceratocystis fimbriata* in solid-state cultures using several agroindustrial wastes (cassava bagasse, apple pomace, amaranth and soybean), and found that the medium with cassava bagasse, apple pomace or soybean produced a strong fruity aroma. Soares *et al.* (*169*) also reported the production of strong pineapple aroma when SSF was carried out using coffee husk as a substrate by this strain. Compounds such as acetaldehyde, ethanol, ethyl acetate (the major compound produced), ethyl isobutyrate, isobutyl acetate, isoamyl acetate and ethyl-3-hexanoate were identified in the headspace of the cultures. The addition of leucine increased ethyl acetate and isoamyl acetate production, and then a strong odour of banana was detected. Bramorski *et al.* (*173*) and Christen *et al.* (*174*) described the production of volatile compounds such as acetaldehyde and 3-methylbutanol by the edible fungus *Rhizopus oryzae* during SSF on tropical agroindustrial substrates.

The production of 6-pentyl-α-pyrone (6-PP), an unsaturated lactone with a strong coconut-like aroma, was studied using liquid and solid substrates by de Araujo *et al.* (*197*). Sugarcane bagasse was adequate for growth and aroma production; it has been demonstrated that, by solid-state fermentation process, it is possible to produce 6-PP at higher concentration than that reported in literature for submerged process.

Kluyveromyces marxianus produced fruity aroma compounds in SSF using cassava bagasse or giant palm bran (*Opuntia ficus indica*) as a substrate (*198*). In this report, several parameters were studied such as initial substrate pH, addition of glucose, cultivation temperature, initial substrate moisture and inoculum size. The analysis showed the production of nine and eleven compounds from palm bran and cassava bagasse substrate, respectively, including alcohols, esters and aldehydes. In both cases, two species remained unidentified, and ethyl acetate, ethanol and acetaldehyde were the major compounds produced. Similar aroma composition was detected in solid-state cultures of *K. marxianus* in a packed bed column bioreactor. Cassava bagasse was found to be a suitable substrate for this yeast culture in the bioreactor. The influence of the aeration rate on the production of volatiles and the correlation between growth and volatile compound generation were demonstrated (*165*).

SSF was found to be very suitable for the production of pyrazines. Besson *et al.* (*170*) and Larroche *et al.* (*171*) studied the biosynthesis of 2,5-dimethylpyrazine (2,5-DMP) and tetramethylpyrazine (TMP) using SSF cultures of *Bacillus subtilis* on soybeans. Production of dairy flavour compounds, such as butyric acid, lactic acid and diacetyl in mixed cultures of *Lactobacillus acidophilus* and *Pediococcus pentosaceus* growing on a semisolid maize-based culture, has been reported (*164*). Soccol *et al.* (*199*) studied the synthesis of lactic acid by *Rhizopus oryzae* in SSF with sugarcane bagasse as a support. They obtained a slightly higher productivity than in submerged cultivation. Moreover, lactic acid production by lactic acid bacteria *Lactobacillus paracasei* and *Lactobacillus amylophilus* GV6 under SSF conditions using sweet sorghum and wheat bran as both support and substrate, respectively, have been investigated (*200–202*).

It is known that several methylketones such as 2-undecanone, 2-nonanone and 2-heptanone are produced at commercial scale by SSF from *Aspergillus niger* using coconut fat as substrate with a yield of 40 % (*3*). Several methods have been developed in order to enable vanillin and furanone or pyranone derivatives of natural origin to be produced from agricultural wastes (Fig. 2). The basic process combines enzyme degradation of plant cell

Table 3. Solid-state fermentation applications in food aroma production

Substrates	Microorganisms	Product	References
		Aroma compounds	
Semisolid maize	*Pediococcus pentosaceus* *Lactobacillus acidophilus*	Butter flavour	(*164*)
Cassava bagasse and giant palm bran	*Kluyveromyces marxianus*	Fruity aroma	(*165*)
Cassava bagasse, apple pomace, amaranth and soybean	*Ceratocystis fimbriata*	Fruity aroma	(*166*)
Pre-gelatinized rice	*Neurospora* sp.	Fruity aroma	(*167*)
Miso	*Zygosaccharomyces rouxii*	HEMF	(*168*)
Coffee husk	*Ceratocystis fimbriata*	Pineapple aroma	(*169*)
Soybeans	*Bacillus subtilis*	Pyrazine	(*170,171*)
Rice koji	*Aspergillus oryzae*	Volatile compounds	(*172*)
Tropical agroindustrial substrates	*Rhizopus oryzae*	Volatile compounds	(*173,174*)
		Enzymes	
Coconut coir pith	*Aspergillus niger*	β-glucosidase	(*175*)
Cranberry pomace	*Lentinus edodes*	β-glucosidase	(*176*)
Wheat straw	*Neurospora crassa*	β-glucosidase	(*177*)
Sugar beet pulp	*Aspergillus niger*	Feruloyl esterase	(*178*)
Wheat bran, maize bran, rice bran and sugarcane bagasse	*Aspergillus flavipes* *Phanerochaete* sp. *Trametes* sp.	Feruloyl esterase	(*79*)
Corn cobs	*Sporotrichum thermophile*	Feruloyl esterase, and *p*-coumaroyl esterase	(*179*)
Wheat bran, rice husk, saw dust, coconut oil cake	*Vibrio costicola*	L-glutaminase	(*180*)
Olive cake and sugar cane bagasse	*Rhizopus rhizopodiformis* *Rhizomucor pusillus*	Lipase	(*181*)
Babassu oil cake	*Penicillium restrictum*	Lipase	(*182*)
Barley bran, triturated nut	*Yarrowia lipolytica*	Lipase	(*183*)
Coconut oil cake	*Candida rugosa*	Lipase	(*184*)
Coconut oil cake, groundnut and sesame, bombay rawa, soya beans, wheat rawa	*Aspergillus* sp.	Lipase	(*185*)
Gingelly oil cake	*Aspergillus niger*	Lipase	(*186*)
GYP medium	*Aspergillus oryzae*	Lipase	(*187*)
Peanut press-cake	*Neurospora sitophila* *Rhizopus oligosporus*	Lipase	(*188*)
Soy cake	*Penicillium simplicissimum*	Lipase	(*189*)
Vegetable oil refinery residue	*Penicillium citrinum*	Lipase	(*190*)
Wheat bran	*Penicillium candidum*	Lipase	(*191*)
Wheat straw	*Penicillium pinophilum*	Phenolic acid esterase	(*192*)
Green gram husk	*Bacillus circulans*	Protease	(*193*)
Soy cake	*Bacillus subtilis*	Protease	(*194*)
Wheat bran, rice husk, rice bran, spent brewing grain, coconut oil cake, palm kernel cake, sesame oil cake, jackfruit seed powder and olive oil cake	*Aspergillus oryzae*	Protease	(*195*)

walls and fungal fermentations. Ferulic acid (precursor) is released from agricultural wastes using polysaccharide-degrading enzymes and specific ferulic acid esterases. Then, ferulic acid is directly converted to vanillin by a selected basidiomycete, *Pycnoporus cinnabarinus*, or by a two-step process using first *Aspergillus niger* to transform the ferulic acid into vanillic acid, then *Pycnoporus cinnabarinus* to obtain vanillin from vanillic acid. Several wastes such as beet pulp and cereal bran (maize, wheat) have been examined (*79,203*).

The usefulness of SSF in the field of food aroma manufacturing does not only concern the direct microbial synthesis of these compounds, but also the production of biocatalysts that could be used in alternative enzy-

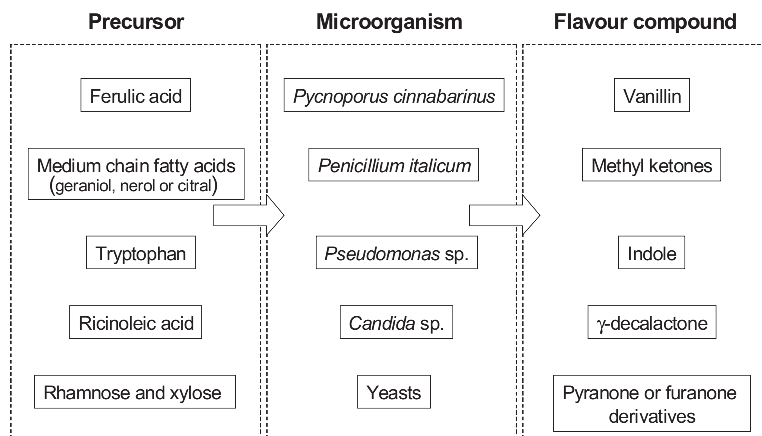

Fig. 2. Examples of flavours produced by microbial bioconversion of the precursors

matic synthesis methods (Table 3). One of the limitations for extensive industrial application of microbial enzymes is their cost, which is determined by the production yields, downstream processing requirements and enzyme stability (*204*). Therefore, it is of interest to increase the productivity of the fermentation processes by optimisation of culture conditions. Since the raw materials employed in the culture medium contribute to the total production costs (*205*), the reduction in the substrate expenditure would be a suitable strategy to increase the productivity of the process. SSF has been shown to be particularly suitable for the production of enzymes by filamentous fungi, since it reproduces the natural living conditions for such microorganisms. Thus, SSF holds tremendous potential for the production of enzymes. Viniegra-González *et al.* (*206*) compared the productivity of the production for three fungal enzymes, using SSF and SmF techniques. They reported that the higher titres found in SSF, compared to SmF, were due to the fact that SSF cultivation works as a fed batch culture with fast oxygenation but slow sugar supply and, in addition, has the added advantage of being a static process without mechanical energy expenditures.

As previously described, lipolytic enzymes are outstanding in terms of their applicability to flavour production (*i.e.* esters). In recent years, increasing attention has been paid to the conversion of processing industry wastes into lipase by solid-state cultures. There are several reports dealing with extracellular lipase production by fungi such as *Rhizopus* sp., *Aspergillus* sp. and *Penicillium* sp. on different solid substrates such as gingelly oil cake, babassu oil cake, olive cake, soy cake and sugar cane bagasse (*181,182,185,186,189,190,207)*. Comparative studies between SmF and SSF systems for lipase production by fungi (*187,207,208*) showed that enzyme yields were higher and more stable in SSF. Moreover, Castilho *et al.* (*205*) performed a detailed economic analysis of the production of *Penicillium restrictum* lipase in both SmF and SSF. This study pointed out that the great advantage of SSF processes resides in their low cost. Recently, this research group has determined the cultivation conditions and operation of tray and packed-bed bioreactors for lipase production by *Penicillium simplicissimum* under SSF using babassu cake as a basal medium (*209*). Maximum lipase activities obtained using packed-bed bioreactors and molasses-supplemented medium were 30 % higher than in tray-type reactor.

Few researchers, though, have investigated the synthesis of lipases by yeasts using this culture mode. Amongst them, Bhusan *et al.* (*210*) reported lipase production in SSF system by an alkalophilic yeast strain belonging to *Candida* sp. Rice bran and wheat bran, oiled with different concentrations of rice bran oil, were used as substrates, and the former was found to provide higher lipase yields. Rao *et al.* (*211*) determined that the C/N ratio of the medium is an important parameter for lipase production by the yeast *Candida rugosa* in solid-state culture. Benjamin and Pandey (*184,212–214)* cultivated *C. rugosa* on coconut oil cake for lipase production using SSF and SmF systems, obtaining higher enzyme production in the former. Recently, Domínguez *et al.* (*183*) reported a great potential of food and agroindustrial wastes (ground nut and barley bran) as support-substrates for lipase production in solid-state cultures of the yeast *Yarrowia lipolytica*, since they led to much higher activities than those found using an inert support.

Glucosidases are also relevant enzymes for the production of food flavours. Many fungi have been found to be capable of producing β-glucosidases during solid-state fermentation on lignocellulosic wastes (*215–217*). In an attempt to find a food-grade β-glucosidase capable of efficiently hydrolyzing bound phenolic compounds, Zheng and Shetty (*176*) reported cranberry pomace as a potential substrate for producing food-grade phenolics and fungal β-glucosidase by *Lentinus edodes* during solid-state fermentation. The *L. edodes* β-glucosidase showed good stability and tolerance to low pH and therefore has potential applications in wine and juice processing for aroma and flavour enrichment through enzymatic hydrolysis of glucoside precursors.

Several researchers have investigated the production of proteases by bacteria and fungi in SSF cultures. In a recent report, Sandhya *et al.* (*195*) demonstrated the superiority of SSF over SmF in protease production by *Aspergillus oryzae*. Various agroindustrial residues (wheat bran, rice husk, rice bran, spent brewing grain, coconut oil cake, palm kernel cake, sesame oil cake, jackfruit seed powder and olive oil cake) were tested for protease production, wheat bran being the best substrate. In the opti-

mal conditions the enzyme production in SSF was 3.5-fold higher than in SmF. A few bacterial strains show a high potential for protease production in SSF. Amongst them, Soares *et al.* (*194*) found a high-yield *Bacillus subtilis* protease production by SSF using soy cake as culture medium. The enzyme level obtained was 10-fold and the enzyme productivity 45 % higher than in SmF.

Glutaminase has found application as a flavour-enhancing agent in food industry. Sabu (*218*) reviews the sources, production, structure, properties, and applications of L-glutaminase. Chandrasekaran (*180*) reported L-glutaminase production by SSF using several organic substrates by marine *Vibrio costicola*. Among the tested substrates, wheat bran and rice bran were more suitable for enzyme production in comparison with saw dust, coconut oil cake, and groundnut cake. Moreover, SSF was found to be preferable to SmF for L-glutaminase biosynthesis by *Pseudomonas fluorescens* in terms of yield efficiency, since a 25- to 30-fold increase in enzyme production was obtained.

Conclusions

The goal of this review was to offer a brief presentation of what is known about natural aroma compounds, focusing especially on the latest advances in their production and use in food industry. Currently, most of the aroma compounds are provided by traditional methods as chemical synthesis or extraction from natural sources. Recently, great interest for natural products has pushed the aroma industry to seek new methods to obtain aroma compounds naturally. An alternative route for this natural synthesis is based on microbial biosynthesis or bioconversion. Critical analysis of the literature shows that the production of relevant aroma compounds for the food processing industry by the use of microbial cultures or enzyme preparations offers several advantages over traditional methodologies. Moreover, it has been well established that the use of solid-state fermentation can give higher yields or better product characteristics than submerged fermentation with low economical costs.

References

1. G. Urbach, The flavour of milk and dairy products: II. Cheese: Contribution of volatile compounds, *Int. J. Dairy Technol. 50* (1997) 79–89.

2. I.L. Gatfield, Production of flavour and aroma compounds by biotechnology, *Food Technol. 10* (1988) 110–122.

3. L. Janssens, H.L. de Pooter, E.J. Vandamme, N.M. Schamp, Production of flavours by microorganisms, *Process Biochem. 27* (1992) 195–215.

4. U. Krings, R.G. Berger, Biotechnological production of flavours and fragrances, *Appl. Microbiol. Biotechnol. 49* (1998) 1–8.

5. E.J. Vandamme, W. Soetaert, Bioflavours and fragrances *via* fermentation and biocatalysis, *J. Chem. Technol. Biotechnol. 77* (2002) 1323–1332.

6. M. Aguedo, M.H. Ly, I. Belo, J.A. Teixeira, J.M. Belin, Y. Waché, The use of enzymes and microorganisms for the production of aroma compounds from lipids, *Food Technol. Biotechnol. 42* (2004) 327–336.

7. T.H. Kim, J.H. Shin, H.H. Baek, H. J. Lee, Volatile flavour compounds in suspension culture of *Agastache rugosa* Kuntze (Korean mint), *J. Sci. Food Agric. 81* (2001) 569–575.

8. F. Drawert, R.G. Berger, R. Godelmann, Regioselective biotransformation of valencene in cell suspension cultures of *Citrus* spp., *Plant Cell Rep. 3* (1984) 37–40.

9. G. Suvarnalatha, M.S. Narayan, G.A. Ravishankar, L.V. Venkataraman, Flavour production in plant cell cultures of Basmati rice (*Oryza sativa* L.), *J. Sci. Food Agric. 66* (1994) 439–442.

10. P.M. Townsley, Chocolate from plant cells, *J. Inst. Can. Sci Technol. Aliment. 7* (1972) 76–78.

11. M. Nakao, K. Ono, S. Takio, The effect of calcium on flavanol production in cell suspension culture of *Polygonum hydropiper*, *Plant Cell Rep. 18* (1999) 759–763.

12. C. Ohsumi, T. Hayashi, K. Sano, Formation of allin in the culture tissues of *Allium sativum* oxidation of S-allyl-L-cysteine, *Phytochemistry, 33* (1993) 107–111.

13. K. Nabeta, Y. Ohnishi, T. Hirose, H. Sugisawa, Monoterpene biosynthesis by callus tissues and suspension cells from *Perilla* species, *Phytochemistry, 22* (1983) 423–425.

14. C.L. Prince, M.L. Shuler, Y. Yamada, Altering flavour profiles in onion (*Allium cepa* L.) root cultures through directed biosynthesis, *Biotechnol. Progr. 13* (1997) 506–510.

15. S. Ayabe, H. Takano, T. Fujita, T. Furuya, H. Hirota, T. Takahashi, Triterpenoid biosynthesis in tissue cultures of *Glycyrrhiza glabra* var. *glandulifera*, *Plant Cell Rep. 9* (1990) 181–184.

16. H. Dornenburg, D. Knorr, Production of the phenolic flavour compounds with cultured cells and tissues of *Vanilla planifolia* species, *Food Biotechnol. 10* (1996) 75–92.

17. S. Harlander: Biotechnology for the Production of Flavouring Materials. In: *Source Book of Flavours*, G. Reineccius (Ed.), Chapman and Hall, New York, USA (1994) pp. 151–175.

18. O.M. Sahai: Plant Tissue Culture. In: *Bioprocess Production of Flavour, Fragrances and Color Ingredients*, A. Gabelman (Ed.), Wiley, New York, USA (1994) pp. 239–275.

19. A.H. Scragg: The Production of Aromas by Plant Cell Cultures. In: *Advances in Biochemical Engineering Biotechnology*, *Vol. 55*, P. Scheper (Ed.), Springer, Berlin, Germany (1997) pp. 239–263.

20. S.R. Rao, G.A. Ravishankar, Plant cell cultures: Chemical factories of secondary metabolites, *Biotechnol. Adv. 20* (2002) 101–153.

21. V. Mulabagal, H.S. Tsay, Plant cell cultures – An alternative and efficient source for the production of biologically important secondary metabolites, *Int. J. Appl. Sci. Eng. 2* (2004) 29–48.

22. F. Dicosmo, M. Misawa, Plant cell and tissue culture: Alternatives for metabolite production, *Biotechnol. Adv. 13* (1995) 425–453.

23. S.R. Rao, G.A. Ravishankar, Vanilla flavour: Production by conventional and biotechnological routes, *J. Sci. Food Agric. 80* (2000) 289–304.

24. G. Davidonis, D. Knorr, Callus formation and shoot regeneration in *Vanilla planifolia*, *Food Biotechnol. 5* (1991) 59–66.

25. C. Funk, P. Brodelius, Phenylpropanoid metabolism in suspension cultures of *Vanilla planifolia* Andr. IV. Induction of vanillic acid formation, *Plant Physiol. 99* (1992) 256–262.

26. C. Funk, P. Brodelius, Phenylpropanoid metabolism in suspension cultures of *Vanilla planifolia* Andr. II. Effects of precursor feeding and metabolic inhibitors, *Plant Physiol. 94* (1990) 95–101.

27. L.G. Romagnoli, D. Knorr, Effects of ferulic acid treatment on growth and flavour development of cultured *Vanilla planifolia* cells, *Food Biotechnol. 2* (1988) 93–104.

28. R.J. Westcott, P.S.J. Cheethan, A.J. Barraclough, Use of organized viable vanilla plant aerial roots for the production of natural vanillin, *Phytochemistry, 35* (1994) 135–138.

29. B. Suresh, T. Ritu, G.A. Ravishankar, Vanilla flavour production through biotransformation using *Capsicum frutescens* root cultures, *Biocatal. Biotransform. 21* (2003) 333–340.

30. M.L. Escamilla-Hurtado, S. Valdes-Martinez, J. Soriano-Santos, A. Tomasini-Campocosio, Effect of some nutritional and environmental parameters on the production of diacetyl and on starch consumption by *Pediococcus pentosaceus* and *Lactobacillus acidophilus* in submerged cultures, *J. Appl. Microbiol. 88* (2000) 142–153.

31. A.Z. Ibragimova, D.A. Yakovlev, E.I.V. Gorshkova, Determination of the ratio of diacetyl and acetaldehyde in streptococcic cultures by gas-liquid chromatography, *Molochnaya Promyshlennost*, *2* (1980) 43–45.

32. P. Bratovanova, To the issue of formation of acetoin and diacetyl in dough part manufactured products, *Biotechnol. Biotec. Eq. 15* (2001) 124–127.

33. N. Bassit, C.Y. Boquien, D. Picque, G. Corrieu, Effect of temperature on diacetyl and acetoin production by *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis* CNRZ 483, *J. Dairy Res. 62* (1995) 123–129.

34. R. Medina de Figueroa, G. Cerutti de Guglielmone, I. Benito de Cardenas, G. Oliver, Flavor compound production and citrate metabolism in *Lactobacillus rhamnosus* ATCC 7469, *Milchwissenschaft*, *53* (1998) 617–619.

35. P. Aungpraphapornchai, H.G. Griffin, M.J. Gasson, Cloning, DNA sequence analysis, and deletion of a gene encoding diacetyl-acetoin reductase from *Lactococcus lactis*, *DNA Sequence*, *10* (1999) 163–172.

36. N.M. Carroll, R.P. Ross, S.M. Kelly, Characterization of recombinant acetolactate synthase from *Leuconostoc lactis* NCW1, *Enzyme Microb. Technol. 25* (1999) 61–67.

37. J. Kronlof, M. Linko, Production of beer using immobilized yeast encoding α-acetolactate decarboxylase, *J. Inst. Brew. 98* (1992) 479–491.

38. W.E. Sandine, P.R. Elliker, M.Y. Pack, V.S. Bavisotto, Use of diacetyl reductase to remove diacetyl from beer, *MBAA TQ*, *2* (1965) 155–159.

39. S. Okui, M. Uchiyama, M. Mizugaki, Metabolism of hydroxy fatty acids. I. Metabolic conversion of ricinoleic acid by a certain microorganism to (+)-D-8-hydroxy-*cis*-5-tetradecenoic acid, *J. Biochem. 53* (1963) 265–270.

40. S. Okui, M. Uchiyama, M. Mizugaki, Metabolism of hydroxy fatty acids. II. Intermediates of the oxidative breakdown of ricinoleic acid by genus *Candida*, *J. Biochem. 54* (1963) 536–540.

41. P.S. Dimick, N.J. Walker, S. Patton, Occurrence and biochemical origin of aliphatic lactones in milk fat – A review, *J. Agric. Food Chem. 17* (1969) 649–655.

42. R.P. Collin, A.F. Halim, Characterization of the major aroma constituent of the fungus *Trichoderma viride* (Pers.), *J. Agric. Food Chem. 20* (1972) 437–438.

43. G.F. Kapfer, R.G. Berger, F. Draweti, Production of 4-decanolide by semicontinuous fermentation of *Tyromyces sambuceus*, *Biotechnol. Lett. 11* (1989) 561–566.

44. G. Allegrone, M. Barbeni, R. Cardillo, C. Fuganti, P. Grasselli, A. Miele, A. Pisciotta, On the steric course of the microbial generation of (Z6)-gamma-dodecenolactone from (10R,S) 10-hydroxyoctadeca-(E8,Z12)-dienoic acid, *Biotechnol. Lett. 13* (1991) 765–768.

45. I.L. Gatfield: Biotechnological Production of Natural Flavor Materials. In: *Flavor Chemistry, Thirty Years of Progress*, R. Teranishi, E.L. Wick, I. Hornstein (Eds.), Plenum Press, New York, USA (1999) pp. 211–227.

46. Y. Wache, M. Aguedo, A. Choquet, I.L. Gatfield, J.M. Nicaud, J.M. Belin, Role of β-oxidation enzymes in γ–decalactone production by the yeast *Yarrowia lipolytica*, *Appl. Environ. Microbiol. 67* (2001) 5700–5704.

47. V. Rojas, J.V. Gil, F. Pinaga, P. Manzanares, Studies on acetate ester production by non-*Saccharomyces* wine yeasts, *Int. J. Food Microbiol. 70* (2001) 283–289.

48. S.Q. Liu, R. Holland, V.L. Crow, Esters and their biosynthesis in fermented dairy products: A review, *Int. Dairy J. 14* (2004) 923–945.

49. M. Nardi, C. Fiez-Vandal, P. Tailliez, V. Monnet, The EstA esterase is responsible for the main capacity of *Lactococcus lactis* to synthesize short chain fatty acid esters *in vitro*, *J. Appl. Microbiol. 93* (2002) 994–1002.

50. E.W. Seitz: Fermentation Production of Pyrazines and Terpenoids for Flavour and Fragrances. In: *Bioprocess Production of Flavour, Fragrance, and Colour,* A. Gabeliman (Ed.), Wiley, New York, USA (1994) pp. 95–134.

51. A.L. Demain, M. Jackson, N.R. Trenner, Thiamine-dependent accumulation of tetramethylpyrazine accompanying a mutation in the isoleucine-valine pathway, *J. Bacteriol*. *94* (1967) 323–326.

52. J. Schindeler, K. Bruns, Process for producing monoterpene containing aroma by fermentation. *German patent 2840143* (1980).

53. W. Bluemke, J. Schrader, Integrated bioprocess for enhanced production of natural flavors and fragrances by *Ceratocystis moniliformis*, *Biomol. Eng. 17* (2001) 137–142.

54. R.S. Dhavlikar, G. Albroscheit, Microbial transformation of terpenoids: Valencene, *Dragoco Report*, *20* (1973) 251–258.

55. E. Pichersky, Y. Iijima, E. Lewinsohn, D.R. Gang, J. Simon, Cloning and sequence of geraniol synthase from sweet basil and use in the production of geraniol and metabolites. *International patent PCT WO 2005060553* (2005).

56. T. Yang, J. Li, H.X. Wang, Y. Zeng, A geraniol synthase gene from *Cinnamomum tenuipilum*, *Phytochemistry*, *66* (2005) 285–293.

57. D.M. Martin, J. Bohlmann, Identification of *Vitis vinifera* (–)-α-terpineol synthase by in silico screening of full-length cDNA ESTs and functional characterization of recombinant terpene synthase, *Phytochemistry*, *65* (2004) 1223–1229.

58. A. Mallouchos, M. Komaitis, A. Koutinas, M. Kanellaki, Wine fermentations by immobilized and free cells at different temperatures. Effect of immobilization and temperature on volatile by-products, *Food Chem. 80* (2002) 109–113.

59. K. Kana, M. Kanellaki, A.A. Koutinas, Volatile by-products formed in batch alcoholic fermentations: Effect of γ-alumina and kissiris supported biocatalysts, *Food Biotechnol. 6* (1992) 65–74.

60. K. Nomura, H. Ogura, Y. Imanishi, Direct synthesis of 2-phenylethanol by hydrogenation of methyl phenylacetate using homogeneous ruthenium-phosphine catalysis under low hydrogen pressure, *J. Mol. Catal. A: Chem. 166* (2001) 345–349.

61. C.E. Fabre, P.J. Blanc, G. Goma, Production of 2-phenylethyl alcohol by *Kluyveromyces marxianus*, *Biotechnol Progr. 14* (1998) 270–274.

62. D. Stark, T. Münch, B. Sonnleitner, I.W. Marison, U. von Stockar, Extractive bioconversion of 2-phenylethanol from L-phenylalanine by *Saccharomyces cerevisiae*, *Biotechnol. Progr. 18* (2002) 514–523.

63. D. Stark, D. Zala, T. Münch, B. Sonnleitner, I.W. Marison, U. von Stockar, Inhibition aspects of the bioconversion of L-phenylalanine to 2-phenylethanol by *Saccharomyces cerevisiae*, *Enzyme Microb. Technol. 32* (2003) 212–223.

64. D. Serp, U. von Stockar, I.W. Marison, Enhancement of 2-phenylethanol productivity by *Saccharomyces cerevisiae* in two-phase fed-batch fermentations using solvent immobilization, *Biotechnol. Bioeng. 82* (2003) 103–110.

65. C.E. Fabre, P.J. Blanc, G. Goma, Screening of yeasts producing 2-phenylethanol, *Biotechnol. Tech. 11* (1997) 523–525.

66. C. Wittman, M. Hans, W. Bluemke, Metabolic physiology of aroma-producing *Kluyveromyces marxianus*, *Yeast, 19* (2002) 1341–1363.

67. M.M.W. Etschmann, D. Sell, J. Schrader, Medium optimization for the production of the aroma compound 2-phenylethanol using a genetic algorithm, *J. Mol. Catal. B: Enzym. 29* (2004) 187–193.

68. M.M.W. Etschmann, W. Bluemke, D. Sell, J. Schrader, Biotechnological production of 2-phenylethanol, *Appl. Microbiol. Biotechnol. 99* (2002) 1–8.

69. H. Priefert, J. Babenhorst, A. Steinbuchel, Biotechnological production of vanillin, *Appl. Microbiol. Biotechnol. 56* (2001) 296–314.

70. N.J. Walton, A. Narbad, C.B. Faulds, G. Williamson, Novel approaches to biosynthesis of vanillin, *Curr. Opin. Biotechnol. 11* (2000) 490–496.

71. Y. Washisu, A. Tetsushi, N. Hashimoto, T. Kanisawa, Manufacture of vanillin and related compounds with *Pseudomonas. Japanese patent JP52279* (1993).

72. J. Overhage, H. Priefert, J. Rabenhorst, A. Steinbuchel, Biotransformation of eugenol to vanillin by a mutant of *Pseudomonas* sp. strain HR199 constructed by disruption of the vanillin dehydrogenase (vdh) gene, *Appl. Microbiol. Biotechnol. 52* (1999) 820–828.

73. S.R. Rao, G.A. Ravishankar, Biotransformation of isoeugenol to vanilla flavour metabolites and capsaicin in suspended and immobilized cell cultures of *Capsicum frutescens*: Study of the influence of β-cyclodextrin and fungal elicitor, *Process Biochem. 35* (1999) 341–348.

74. E. Shimoni, U. Ravid, Y. Shoham, Isolation of a *Bacillus* sp. capable of transforming isoeugenol to vanillin, *J. Biotechnol. 78* (2000) 1–9.

75. E. Shimoni, T. Baasov, U. Ravid, Y. Shoham, Biotransformations of propenylbenzenes by an *Arthrobacter* sp. and its *t*-anethole blocked mutants, *J. Biotechnol. 105* (2003) 61–70.

76. L. Lesage-Meessen, M. Delattre, M. Haon, J.F. Thibault, B. Colonna Ceccaldi, P. Brunerie, M. Asther, A two-step bioconversion process for vanillin production from ferulic acid combining *Aspergillus niger* and *Pycnoporus cinnabarinus*, *J. Biotechnol. 50* (1996) 107–113.

77. L. Lesage-Meessen, A. Lomascolo, E. Bonnin, J.F. Thibault, A. Buleon, M. Roller, M. Asther, E. Record, B.C. Ceccaldi, M. Asther, A biotechnological process involving filamentous fungi to produce natural crystalline vanillin from maize bran, *Appl. Biochem. Biotechnol. 102* (2002) 141–153.

78. E. Bonnin, H. Grange, L. Lesage-Meessen, M. Asther, J.F. Thibault, Enzymic release of cellobiose from sugar beet pulp, and its use to flavour vanillin production in *Pycnoporus cinnabarinus* from vanillic acid, *Carbohydr. Polym. 41* (2000) 143–151.

79. S. Mathew, T.E. Abraham, Studies on the production of feruloyl esterase from cereal brans and sugar cane bagasse by microbial fermentation, *Enzyme Microb. Technol. 36* (2005) 565–570.

80. A. Narbad, M.J. Gasson, Metabolism of ferulic acid via vanillin using a novel CoA-dependent pathway in a newly-isolated strain of *Pseudomonas fluorescens*, *Microbiology, 144* (1998) 1397–1405.

81. B. Falconnier, C. Lapierre, L. Lesage-Meessen, G. Yonnet, P. Brunerie, B. Colonna-Ceccaldi, G. Corrieu, M. Asther, Vanillin as a product of ferulic acid biotransformation by the white-rot fungus *Pycnoporus cinnabarinus* I-937: Identification of metabolic pathways, *J. Biotechnol. 37* (1994) 123–132.

82. G.S. Clark, Benzaldehyde. An aroma chemical profile, *Perfum. Flavor. 20* (1995) 53–60.

83. A. Lomascolo, L. Lesage-Meessen, M. Labat, D. Navarro, M. Delattre, M. Asther, Enhanced benzaldehyde formation by a monokaryotic strain of *Pycnoporus cinnabarinus* using a selective solid adsorbent in the culture medium, *Can. J. Microbiol. 45* (1999) 653–657.

84. S.D. Geusz, D.M. Anderson, Process of using bacteria that metabolize phenylacetate through mandelate. *US patent 5 151 353* (1992).

85. R. Wilcocks, O.P Ward, Factors affecting 2-hydroxypropiophenone formation by benzoylformate decarboxylase from *Pseudomonas putida*, *Biotechnol. Bioeng. 39* (1992) 1058–1063.

86. R. Wilcocks, O.P Ward, C. Scott, J.D. Nolan, H. Yaping, P. Elizabeth, Acyloin formation by benzoylformate decarboxylase from *Pseudomonas putida*, *Appl. Environ. Microbiol. 58* (1992) 1699–1704.

87. T. Kawabe, H. Morita, Production of benzaldehyde and benzyl alcohol by the mushroom *Polyporus tuberaster* K2606, *J. Agric. Food Chem. 42* (1994) 2556–2560.

88. K.A. Jensen, K.M.C. Evans, T.K. Kirk, K.E. Hammel, Biosynthetic pathway for veratryl alcohol in the ligninolytic fungus *Phanerochaete chrysosporium*, *Appl. Environ. Microbiol. 60* (1994) 709–714.

89. C. Lapadatescu, C. Ginies, A. Djian, H.E. Spinnler, J.L. Le Quéré, P. Bonnarme, Regulation of the synthesis of aryl metabolites by phospholipids sources in the white-rot fungus *Bjerkandera adusta*, *Arch. Microbiol. 171* (1999) 151–158.

90. A. Lomascolo, M. Asther, D. Navarro, C. Antona, M. Delattre, L. Lesage-Meessen, Shifting the biotransformation pathways of L-phenylalanine into benzaldehyde by *Trametes suaveolens* CBS 334.85 using HP20 resin, *Lett. Appl. Microbiol. 32* (2001) 262–267.

91. J.K. Park, J.Y. Jung, Production of benzaldehyde by encapsulated whole-cell benzoylformate decarboxylase, *Enzyme Microb. Technol. 30* (2002) 726–733.

92. H.T. Badings, J.J.G. Van der Pol, R. Neeter: Aroma Compounds which Contribute to the Difference in Flavour Between Pasteurized Milk and UHT Milk. In: *Flavour '81*, P. Schreier (Ed.), Walter de Gruyter, New York, USA (1981) pp. 683–692.

93. L. Moio, P. Etievant, D. Langlois, J. Dekimpe, F. Addeo, Detection of powerful odorants in heated milk by use of extract dilution sniffing analysis, *J. Dairy Res. 61* (1994) 385–394.

94. S. Hagedorn, B. Kaphammer, Microbial biocatalysis in the generation of flavor and fragrance chemicals, *Annu. Rev. Microbiol. 48* (1994) 773–800.

95. J.S. Dordick, Enzymic catalysis in monophasic organic solvents, *Enzyme Microb. Technol. 11* (1989) 194–211.

96. R. Kumar, J. Modak, G. Madras, Effect of chain length of the acid on the enzymatic synthesis of flavours in supercritical carbon dioxide, *Biochem. Eng. J. 23* (2005) 199–202.

97. A. Larios, H.S. Garcia, M.R. Oliart, G. Valerio-Alfaro, Synthesis of flavour and fragrance esters using *Candida antarctica* lipase, *Appl. Microbiol. Biotechnol. 65* (2004) 373–376.

98. S. Srivastava, J. Modak, G. Madras, Enzymatic synthesis of flavours in supercritical carbon dioxide, *Ind. Eng. Chem. Res. 41* (2002) 1940–1945.

99. G.A. Macedo, M.M.S. Lozano, G.M. Pastore, Enzymatic synthesis of short chain citronellyl esters by a new lipase from *Rhizopus* sp., *Electron. J. Biotechnol. 6* (2003) 72–75.

100. D.Y. Kwon, Y.J. Hong, S.H. Yoon, Enantiomeric synthesis of (S)-2-methylbutanoic acid methyl ester, apple flavour, using lipases in organic solvent, *J. Agric. Food Chem. 48* (2000) 524–530.

101. Y. Shimada, Y. Hirota, T. Baba, S. Kato, A. Sugihara, S. Moriyama, Y. Tominaga, T. Terai, Enzymatic synthesis of steryl esters of polyunsaturated fatty acids, *J. Am. Oil Chem. Soc. 76* (1999) 1139–1142.

102. W. Chulalaksananukul, J.S. Condoret, D. Combes, Kinetics of geranyl acetate synthesis by lipase-catalyzed transesterification in *n*-hexane, *Enzyme Microb. Technol. 14* (1992) 293–298.

103. W. Chulalaksananukul, J.S. Condoret, D. Combes, Geranyl acetate synthesis by lipase-catalyzed transesterification in supercritical carbon dioxide, *Enzyme Microb. Technol. 15* (1993) 691–698.

104. S.Y. Huang, H.L. Chang, M. Goto, Preparation of surfactant-coated lipase for the esterification of geraniol and acetic acid in organic solvents, *Enzyme Microb. Technol. 22* (1998) 552–557.

105. S.Y. Huang, H.L. Chang, Kinetic study on the esterification of geraniol and acetic acid in organic solvents using surfactant-coated lipase, *J. Chem. Technol. Biotechnol. 74* (1999) 183–187.

106. A. Zaidi, J.L. Gainer, G. Carta, A. Mirani, T. Kadiri, Y. Belarbi, Esterification of fatty acids using nylon-immobilized lipase in *n*-hexane: Kinetics parameters and chain-length effects, *J. Biotechnol. 93* (2000) 209–216.

107. S.H. Krishna, B. Manohar, S. Divakar, S.G. Prapulla, N.G. Karnath, Optimization of isoamyl acetate production by using immobilized lipase from *Mucor miehei* by response surface methodology, *Enzyme Microb. Technol. 26* (2000) 131–136.

108. J. Kim, D.H. Altreuter, D.S. Clark, J.S. Dordick, Rapid synthesis of fatty acid esters for use as potential food flavours, *J. Am. Oil Chem. Soc. 75* (1998) 1109–1113.

109. F. Laboret, R. Perraud, Lipase-catalyzed production of short-chain acids terpenyl esters of interest to the food industry, *Appl. Biochem. Biotechnol. 82* (1999) 185–198.

110. F.W. Welsh, R.E. Williams, K.H. Dawson, Lipase-mediated synthesis of low-molecular-weight flavour esters, *J. Food Sci. 55* (1990) 1679–1682.

111. G. Langrand, N. Rondot, C. Triantaphylides, J. Baratti, Short chain flavour esters synthesis by microbial lipases, *Biotechnol. Lett. 12* (1990) 581–586.

112. M. Norin, J. Boutelje, E. Holmberg, K. Hult, Lipase immobilized by adsorption: Effect of support hydrophobicity on the reaction rate of ester synthesis in cyclohexane, *Appl. Microbiol. Biotechnol. 28* (1988) 527–530.

113. E. Wehtje, J. Kaur, P. Adlercreutz, S. Chand, B. Mattiasson, Water activity control in enzymatic esterification processes, *Enzyme Microb. Technol. 21* (1997) 502–510.

114. P. Mensah, J.L. Gainer, G. Carta, Adsorptive control of water in esterification with immobilized enzymes: I. Batch reactor behaviour, *Biotechnol. Bioeng. 60* (1998) 434–444.

115. A. van der Padt, J.J.W. Sewalt, K. Van't Riet, Online water removal during enzymatic triacylglycerol synthesis by means of pervaporation, *J. Membr. Sci. 80* (1993) 199–208.

116. J.C. Jeong, S.B. Lee, Enzymatic esterification reaction in organic media with continuous water stripping: Effect of water content on reactor performance and enzyme agglomeration, *Biotechnol. Tech. 11* (1997) 853–858.

117. L. Gubicza, A. Kabiri-Badr, E. Keoves, K. Belafi-Bako, Large-scale enzymatic production of natural flavour esters in organic solvent with continuous water removal, *J. Biotechnol. 84* (2000) 193–196.

118. A.P. Ison, A.R. Macrae, C.G. Smith, J. Bosley, Mass transfer effects in solvent-free fat interesterification reactions: Influences on catalyst design, *Biotechnol. Bioeng. 43* (1994) 122–130.

119. N. Goma-Doncescu, M.D. Legoy, Original transesterification route for fatty acid ester production from vegetable oils in a solvent-free system, *J. Am. Oil Chem. Soc. 74* (1997) 1137–1143.

120. B.K. De, D.K. Bhattacharyya, C. Bandhu, Enzymatic synthesis of fatty alcohol esters by alcoholysis, *J. Am. Oil Chem. Soc. 76* (1999) 451–453.

121. M. Karra-Chaabouni, S. Pulvin, D. Touraud, D. Thomas, Parameters affecting the synthesis of geranyl butyrate by esterase 30,000 from *Mucor miehei*, *J. Am. Oil Chem. Soc. 75* (1998) 1201–1206.

122. Z.R. Yu, S.S.H. Rizvi, J.A. Zollweg, Enzymic esterification of fatty acid mixtures from milk fat and anhydrous milk fat with canola oil in supercritical carbon dioxide, *Biotechnol. Progr. 8* (1992) 508–513.

123. R.C. Chang, S.J. Chou, J.F. Shaw, Synthesis of fatty acid esters in recombinant *Staphylococcus epidermidis* lipases in aqueous environment, *J. Agric. Food Chem. 49* (2001) 2619–2622.

124. L.M.L. Bruno, J.L.M. Filho, E.H. Melo, H.F. De Castro, Ester synthesis catalyzed by *Mucor miehei* lipase immobilized on magnetic polysiloxane-polyvynil alcohol particles, *Appl. Biochem. Biotechnol. 113* (2004) 189–199.

125. J.P. Chen, Y.N. Hwang, Polyvinyl formal resin plates impregnated with lipase-entrapped sol-gel polymer for flavour ester synthesis, *Enzyme Microb. Technol. 33* (2003) 513–519.

126. E. Alvarez-Macarie, J. Baratti, Short chain flavour ester synthesis by a new esterase from *Bacillus licheniformis*, *J. Mol. Catal. B: Enzym. 10* (2000) 377–383.

127. A. Domínguez, A. Sanromán, P. Fuciños, M.L. Rúa, L. Pastrana, M.A. Longo, Quantification of intra- and extra-cellular thermophilic lipase/esterase production by *Thermus* sp., *Biotechnol. Lett. 26* (2004) 705–708.

128. P. Fuciños, A. Domínguez, M.A. Sanromán, M.A. Longo, M.L. Rúa, L. Pastrana, Production of thermostable lipolytic activity by *Thermus* species, *Biotechnol. Progr. 21* (2005) 1198–1205.

129. P. Fuciños, C.M. Abadín, A. Sanromán, M.A. Longo, L. Pastrana, M.L. Rúa, Identification of extracellular lipases/esterases produced by *Thermus thermophilus* HB27: Partial purification and preliminary biochemical characterisation, *J. Biotechnol. 117* (2005) 233–241.

130. T. Nishimura, H. Kato, Taste of free amino acids and peptides, *Food Rev. Int. 4* (1988) 175–194.

131. K. Maehashi, M. Matsuzaki, Y. Yamamoto, S. Udaka, Isolation of peptides from an enzymatic hydrolysate of food proteins and characterization of their taste properties, *Biosci. Biotechnol. Biochem. 63* (1999) 555–559.

132. J.W. Sawhill, Production of whey protein hydrolyzates using fungal proteases. *US patent 6787168* (2004).

133. D.E. Stevenson, D.J. Ofman, G.A. Fenton, Protease-catalysed condensation-oligomerisation of hydrophobic peptides as a means of flavour modification, *J. Mol. Catal. B: Enzym. 5* (1998) 39–44.

134. H.H. Baek, K.R. Cadwallader, Volatile compounds in flavour concentrates produced from crayfish-processing by-products with and without protease treatment, *J. Agric. Food Chem. 44* (1996) 3262–3267.

135. M.D. Aaslyng, J.S. Elmore, D.S. Mottram, Comparison of the aroma characteristics of acid-hydrolyzed and enzyme-hydrolyzed vegetable proteins produced from soy, *J. Agric. Food Chem. 46* (1998) 5225–5231.

136. I. Gill, R. Lopez-Fandiño, X. Jorba, E.N. Vulfson, Biologically active peptides and enzymatic approaches to their production, *Enzyme Microb. Technol. 18* (1996) 162–183.

137. A. Matsushita, S. Ozaki, Purification and sequence determination of tasty tetrapeptide (Asp-Asp-Asp-Asp) from beer yeast and its enzymic synthesis, *Pept. Chem. 32* (1995) 249–252.

138. X. Jorba, I. Gill, E.N. Vulfson, Enzymatic synthesis of the delicious peptide fragments in eutectic mixtures, *J. Agric. Food Chem. 43* (1995) 2356–2541.

139. K.N. Kilcawley, M.G. Wilkinson, P.F. Fox, Enzyme modified cheese, *Int. Dairy J. 8* (1998) 1–10.

140. S.S. Haileselassie, B.H. Lee, B.F. Gibbs, Purification and identification of potentially bioactive peptides from enzyme-modified cheese, *J. Dairy Sci. 82* (1999) 1612–1617.

141. A.P. Aryan, B. Wilson, C.R. Strauss, P.J. Williams, The properties of glycosidases of *Vitis vinifera* and a comparison of their β-glucosidase activity with that of exogenous enzymes. An assessment of possible applications in enology, *Am. J. Enol. Vitic. 38* (1987) 182–188.

142. B.M. de Roode, L. Oliehoek, A. Van der Padt, M.C.R. Franssen, R.M. Boom, Downstream processing of enzymatically produced geranyl glucoside, *Biotechnol. Progr. 17* (2001) 881–886.

143. M.C.R. Franssen, L. Alessandrini, G. Terraneo, Biocatalytic production of flavors and fragrances, *Pure Appl. Chem. 77* (2005) 273–279.

144. D.M. de Temiño, W. Hartmeier, M.B. Ansorge-Schumacher, Entrapment of the alcohol dehydrogenase from *Lactobacillus kefir* in polyvinyl alcohol for the synthesis of chiral hydrophobic alcohols in organic solvents, *Enzyme Microb. Technol. 36* (2005) 3–9.

145. J.W. de Kraker, M. Schurink, M.C.R. Franssen, W.A. Konig, A. de Groot, H.J. Bouwmeester, Hydroxylation of sesquiterpenes by enzymes from chicory (*Cichorium intybus* L.) roots, *Tetrahedron, 59* (2003) 409–418.

146. A. Yoshida, Y. Takenaka, H. Tamaki, I. Frebort, O. Adachi, H. Kumagai, Vanillin formation by microbial amine oxidases from vanillylamine, *J. Ferment. Bioeng. 84* (1997) 603–605.

147. R. Nandakumar, K. Yoshimune, M. Wakayama, M. Moriguchi, Microbial glutaminase: Biochemistry, molecular approaches and applications in the food industry, *J. Mol. Catal. B: Enzym. 23* (2003) 87–100.

148. A. Pandey, Recent process developments in solid-state fermentation, *Process Biochem. 27* (1992) 109–117.

149. A. Pandey, C.R. Soccol, D. Mitchell, New developments in solid state fermentation: I Bioprocesses and products, *Process Biochem. 35* (2000) 1153–1169.

150. A. Pandey, P. Selvakumar, C.R. Soccol, P. Nigam, Solid state fermentation for the production of industrial enzymes, *Curr. Sci. 77* (1999) 149–162.

151. G. Feron, P. Bonnarme, A. Durand, Prospects of the microbial production of food flavours, *Trends Food Sci. Technol. 7* (1996) 285–293.

152. M.R. Johns, D.M. Stuart, Production of pigments by *Monascus purpureus* in solid culture, *J. Ind. Microbiol. 8* (1991) 23–28.

153. T. Robinson, P. Nigam, Bioreactor design for protein enrichment of agricultural residues by solid state fermentation, *Biochem. Eng. J. 13* (2003) 197–203.

154. D.A. Mitchell, N. Krieger, D. Stuart, A. Pandey, New developments in solid-state fermentation II. Rational approaches to the design, operation and scale-up of bioreactors, *Process Biochem. 35* (2000) 1211–1225.

155. F.J. Nagel, J. Tramper, M.S. Bakker, A. Rinzema, Temperature control in a continuously mixed bioreactor for solid-state fermentation, *Biotechnol. Bioeng. 72* (2001) 219–230.

156. D.M. Stuart, D.A. Mitchell, M.R. Johns, J.D. Litster, Solid-state fermentation in rotating drum bioreactors: Operating variables affect performance through their effects on transport phenomena, *Biotechnol. Bioeng. 63* (1999) 383–391.

157. I. Rivela, S. Rodríguez Couto, A. Sanromán, Extracellular ligninolytic enzyme production by *Phanerochaete chrysosporium* in a new solid-state bioreactor, *Biotechnol. Lett. 22* (2000) 1443–1447.

158. J. Echevarria, J.A.R. Leon, M.E. Espinosa, G. Delgado, Optimization of solid state fermentation of sugarcane by *Aspergullus niger* considering the particle size effect, *Acta Biotechnol. 11* (1991) 15–22.

159. J. Barrios-Gonzalez, H. Gonzalez, A. Mejia, Effect of particle size, packing density and agitation on penicillin production in solid state fermentation, *Biotechnol. Adv. 11* (1993) 539–547.

160. L.M. Pastrana, M.P. Gonzalez, J. Pintado, M.A. Murado, Interactions affecting gibberellic acid production in solid-state culture: A factorial study, *Enzyme Microb. Technol. 17* (1995) 784–790.

161. B.L. Liu, Y.M. Tzeng, Water content and water activity for the production of cyclodepsipeptide in solid state fermentation, *Biotechnol. Lett. 21* (1999) 657–661.

162. R.G. Berger: *Aroma Biotechnology*, Springer, Berlin, Germany (1995).

163. C.R. Soccol, L.P.S. Vandenberghe, Overview of applied solid-state fermentation in Brazil, *Biochem. Eng. J. 13* (2003) 205–218.

164. M.L. Escamilla-Hurtado, S.E. Valdes-Martinez, J. Soriano-Santos, R. Gomez-Pliego, J.R. Verde-Calvo, A. Reyes-Dorantes, A. Tomasini-Campocosio, Effect of culture conditions on production of butter flavor compounds by *Pediococcus pentosaceus* and *Lactobacillus acidophilus* in semisolid maize-based cultures, *Int. J. Food Microbiol. 105* (2005) 305–316.

165. A. Medeiros, A. Pandey, P. Christen, P.S.G. Fontoura, R.J.S. Freitas, C.R. Soccol, Aroma compounds produced by *Kluyveromyces marxianus* in solid-state fermentation on packed bed column bioreactor, *World J. Microbiol. Biotechnol. 17* (2001) 767–771.

166. A. Bramorski, P. Christen, M. Ramirez, C.R. Soccol, S. Revah, Production of volatile compounds by the edible fungus *Rhizopus oryzae* during solid-state cultivation on tropical agro-industrial substrates, *Biotechnol. Lett. 20* (1998) 359–362.

167. G.M. Pastore, Y.K. Park, D.B. Min, Production of a fruity aroma by *Neurospora* from beiju, *Mycol. Res. 98* (1994) 25–35.

168. E. Sugawara, S. Hashimoto, Y. Sakurai, A. Kobayashi, Formation by yeast of the HEMF (4-hydroxy-2 (or 5)-ethyl-5 (or 2)-methyl-3 (2H)-furanone) aroma components in Miso with aging, *Biosci. Biotechnol. Biochem. 58* (1994) 1134–1135.

169. M. Soares, P. Christen, A. Pandey, C.R. Soccol, Fruity flavour production by *Ceratocystis fimbriata* grown on coffee husk in solid state fermentation, *Process Biochem. 35* (2000) 857–861.

170. I. Besson, C. Creuly, J.B. Gros, C. Larroche, Pyrazine production by *Bacillus subtilis* in solid state fermentation on soybeans, *Appl. Microbiol. Biotechnol. 47* (1997) 489–495.

171. C. Larroche, I. Besson, J.B. Gros, High pyrazine production by *Bacillus subtilis* in solid substrate fermentation on ground soy-beans, *Process Biochem. 34* (1999) 67–74.

172. K. Ito, K. Yoshida, T. Ishikawa, S. Kobayashi, Volatile compounds produced by fungus *Aspergillus oryzae* in rice koji and their changes during cultivation, *J. Ferment. Bioeng. 70* (1990) 169–172.

173. A. Bramorski, P. Christen, M. Ramirez, C.R. Soccol, S. Revah, Production of volatile compounds by the edible fungus *Rhizopus oryzae* during solid-state cultivation on tropical agro-industrial substrates, *Biotechnol. Lett. 20* (1998) 359–362.

174. P. Christen, A. Bramorski, S. Revah, C.R. Soccol, Characterization of volatile compounds produced by *Rhizopus* strains grown on agro-industrial solid wastes, *Bioresour. Technol. 71* (2000) 211–215.

175. P.K.A. Muniswaran, P. Selvakumar, N.C.L.N. Charyulu, Production of cellulases from coconut coir pith in solid state fermentation, *J. Chem. Technol. Biotechnol. 60* (1994) 147–151.

176. Z. Zheng, K. Shetty, Solid-state bioconversion of phenolics from cranberry pomace and role of *Lentinus edodes* β-glucosidase, *J. Agric. Food Chem. 48* (2000) 895–900.

177. B.J. Macris, D. Kekos, X. Evangelidou, M. Galiotou-Panayotou, P. Rodis, Solid-state fermentation of straw with *Neurospora crassa* for CMCase and β-glucosidase production, *Biotechnol. Lett. 9* (1987) 661–664.

178. M. Asther, M. Haon, S. Roussos, E. Record, M. Delattre, L. Lesage-Meessen, Feruloyl esterase from *Aspergillus niger*: A comparison of the production in solid state and submerged fermentation, *Process Biochem. 38* (2002) 685–691.

179. E. Topakas, E. Kalogeris, D. Kekos, B.J. Macris, P. Christakopoulos, Production of phenolics from corn cobs by coupling enzymic treatment and solid state fermentation, *Eng. Life Sci. 4* (2004) 283–286.

180. M.J. Chandrasekaran, Industrial enzymes from marine microorganisms: The Indian scenario, *J. Mar. Biotechnol. 5* (1997) 86–89.

181. J. Cordova, M. Nemmaoui, M. Ismaili-Alaoui, A. Morin, S. Roussos, M. Raimbault, B. Benjilali, Lipase production by solid state fermentation of olive cake and sugar cane bagasse, *J. Mol. Catal. B: Enzym. 5* (1998) 75–78.

182. A.K. Gombert, A.L. Pinto, L.R. Castilho, D.M.G. Freire, Lipase production by *Penicillium restrictum* in solid-state fermentation using babassu oil cake as substrate, *Process Biochem. 35* (1999) 85–90.

183. A. Domínguez, M. Costas, M.A. Longo, A. Sanromán, A novel application of solid state culture: Production of lipases by *Yarrowia lipolytica*, *Biotechnol. Lett. 25* (2003) 1225–1229.

184. S. Benjamin, A. Pandey, Coconut cake – A potent substrate for the production of lipase by *Candida rugosa* in solid-state fermentation, *Acta Biotechnol. 17* (1997) 241–251.

185. K. Adinarayana, K.V.V.S.N. Bapi Raju, M. Igbal Zargar, R. Bahvani Devi, P. Jhansi Lakshmi, P. Ellaiah, Optimization of process parameters for production of lipase in solid-state fermentation by newly isolated *Aspergillus* species, *Indian J. Biotechnol. 3* (2004) 65–69.

186. N.R. Kamini, J.G.S. Mala, R. Puvanakrishnan, Lipase production from *Aspergillus niger* by solid-state fermentation using gingelly oil cake, *Process Biochem. 33* (1998) 505–511.

187. K. Ohnishi, Y. Yoshida, J. Sekiguchi, Lipase production of *Aspergillus oryzae*, *J. Ferment. Bioeng. 77* (1994) 490–495.

188. L.R. Beuchat, Flavor chemistry of fermented peanuts, *Ind. Eng. Chem. Prod. Res. Dev. 21* (1982) 533–536.

189. M. Di Luccio, F. Capra, N.P. Ribeiro, G.D.L.P. Vargas, D.M.G. Freire, D. De Oliveira, Effect of temperature, moisture, and carbon supplementation on lipase production by solid-state fermentation of soy cake by *Penicillium simplicissimum*, *Appl. Biochem. Biotechnol. 113* (2004) 173–180.

190. O.A. Miranda, A.A. Salgueiro, M.C.B. Pimentel, J.L. Lima Filho, E.H.M. Melo, N. Duran, Lipase production by a Brazilian strain of *Penicillium citrinum* using an industrial residue, *Bioresour. Technol. 69* (1999) 145–147.

191. E. Ortiz-Vazquez, M. Granados-Baeza, G. Rivera-Muñoz, Effect of culture conditions on lipolytic enzyme production by *Penicillium candidum* in a solid state fermentation, *Biotechnol. Adv. 11* (1993) 409–416.

192. A. Castanares, S.I. McCrae, T.M. Wood, Purification and properties of a feruloyl/p-coumaroyl esterase from the fungus *Penicillium pinophilum*, *Enzyme Microb. Technol. 14* (1992) 875–884.

193. R.S. Prakasham, Ch.S. Rao, R.S. Rao, P.N. Sarma, Alkaline protease production by an isolated *Bacillus circulans* under solid-state fermentation using agroindustrial waste: Process parameters optimization, *Biotechnol. Progr. 21* (2005) 1380–1388.

194. V.F. Soares, L.R. Castilho, E.P.S. Bon, D.M.G. Freire, High-yield *Bacillus subtilis* protease production by solid-state fermentation, *Appl. Biochem. Biotechnol. 121–124* (2005) 311–319.

195. C. Sandhya, A. Sumantha, G. Szakacs, A. Pandey, Comparative evaluation of neutral protease production by *Aspergillus oryzae* in submerged and solid-state fermentation, *Process Biochem. 40* (2005) 2689–2694.

196. A. Bramorski, C.R. Soccol, P. Christen, S. Revah, Fruit aroma production by *Ceratocystis fimbriata* in static cultures from solid agro-industrial wastes, *Rev. Microbiol. 28* (1998) 208–212.

197. A.A. de Araujo, G.M. Pastore, R.G. Berger, Production of coconut aroma by fungi cultivation in solid-state fermentation, *Appl. Biochem. Biotechnol. 98–100* (2002) 747–751.

198. A.B.P. Medeiros, A. Pandey, R.J.S. Freitas, P. Christen, C.R. Soccol, Optimization of the production of aroma compounds by *Kluyveromyces marxianus* in solid-state fermentation using factorial design and response surface methodology, *Biochem. Eng. J. 6* (2000) 33–39.

199. C.R. Soccol, B. Marin, M. Rimbault, J.M. Labeault, Potential of solid state fermentation for production of L(+) lactic acid by *Rhizopus oryzae*, *Appl. Microbiol. Biotechnol. 41* (1994) 286–290.

200. K. Richter, A. Träger, L(+) lactic acid from sweet sorghum by submerged and solid-state fermentations, *Acta Biotechnol. 14* (1994) 367–378.

201. B.J. Naveena, M. Altaf, K. Bhadrayya, S.S. Madhavendra, G. Reddy, Direct fermentation of starch to L(+) lactic acid in SSF by *Lactobacillus amylophilus* GV6 using wheat bran as support and substrate: Medium optimization using RSM, *Process Biochem. 40* (2005) 681–690.

202. B.J. Naveena, M. Altaf, K. Bhadriah, G. Reddy, Selection of medium components by Plackett–Burman design for production of L(+)lactic acid by *Lactobacillus amylophilus* GV6 in SSF using wheat bran, *Bioresour. Technol. 96* (2005) 485–490.

203. E. Bonnin, M. Brunel, Y. Gouy, L. Lesage-Meessen, M. Asther, J.F. Thibault, *Aspergillus niger* I-1472 and *Pycnoporus cinnabarinus* MUCL39533, selected for the biotransformation of ferulic acid to vanillin, are also able to produce cell wall polysaccharide-degrading enzymes and feruloyl esterases, *Enzyme Microb. Technol. 28* (2001) 70–80.

204. L. Kanwar, B.K. Gogoi, P. Goswami, Production of a *Pseudomonas* lipase in *n*-alkane substrate and its isolation using an improved ammonium sulfate precipitation technique, *Bioresour. Technol. 84* (2002) 207–211.

205. L.R. Castilho, C.M.S. Polato, E.A. Baruque, G.L. Sant' Anna, D.M.G. Freire, Economic analysis of lipase production by *Penicillium restrictum* in solid-state and submerged fermentations, *Biochem. Eng. J. 4* (2000) 239–247.

206. G. Viniegra-González, E. Favela-Torres, C.N. Aguilar, J. de Jesús Romero-Gómez, G. Díaz-Godínez, C. Augur, Advantages of fungal enzyme production in solid state over liquid fermentation systems, *Biochem. Eng. J. 13* (2003) 157–167.

207. P. Christen, N. Angeles, G. Corzo, A. Farres, S. Revah, Microbial lipase production on a polymeric resin, *Biotechnol. Tech. 9* (1995) 597–600.

208. G. Rivera-Muñoz, J.R. Tinoco-Valencia, S. Sanchez, A. Farres, Production of microbial lipases in a solid-state fermentation system, *Biotechnol. Lett. 13* (1991) 277–280.

209. M.L.E. Gutarra, E.D.C. Cavalcanti, L.R. Castilho, D.M.G. Freire, G.L. Sant'Anna, Lipase production by solid-state fermentation. Cultivation conditions and operation of tray and packed-bed bioreactors, *Appl. Biochem. Biotechnol. 121* (2005) 105–116.

210. B. Bhusan, N.S. Dosanjih, K. Kumar, G.S. Hoondal, Lipase production from an alkalophilic yeast sp. by solid state fermentation, *Biotechnol. Lett. 16* (1994) 841–842.

211. P.V. Rao, K. Jayaraman, C.M. Lakshmanan, Production of lipase by *Candida rugosa* in solid-state fermentation. 2: Medium optimization and effect of aeration, *Process Biochem. 28* (1993) 391–395.

212. S. Benjamin, A. Pandey, Lipase production by *Candida rugosa* on copra waste extract, *Indian J. Microbiol. 36* (1996) 201–204.

213. S. Benjamin, A. Pandey, Optimization of liquid media for lipase production by *Candida rugosa*, *Bioresour. Technol. 55* (1996) 167–170.

214. S. Benjamin, A. Pandey, Enhancement of lipase production during repeated batch cultivation using immobilised *Candida rugosa*, *Process Biochem. 32* (1997) 437–440.

215. Y.D. Hang, E.E. Woodams, Apple pomace: A potential substrate for production of β-glucosidase by *Aspergillus foetidus*, *Food Sci. Technol. 27* (1994) 587–589.

216. A. Martino, P.G. Pifferi, G. Spagna, Production of β-glucosidase by *Aspergillus niger* using carbon sources derived from agricultural wastes, *J. Chem. Technol. Biotechnol. 60* (1994) 247–252.

217. A. Gupta, D. Madamwar, Solid-state fermentation of lignocellulosic waste for cellulase and β-glucosidase production by cocultivation of *Aspergillus ellipticus* and *Aspergillus fumigatus*, *Biotechnol. Progr. 13* (1997) 166–169.

218. A. Sabu: Microbial Enzymes: Production and Applications: L-glutaminase. In: *Concise Encyclopedia of Bioresource Technology*, A. Pandey (Ed.), Haworth Press, Binghamton, USA (2004) pp. 517–525.

FTB 44 (3) 335-353.

Proizvodnja aromatskih sastojaka hrane: mikrobna i enzimska metodologija

Sažetak

Posljednjih su godina sve veći zahtjevi za prirodnim proizvodima prehrambene industrije ohrabrili nevjerojatne pokušaje razvoja biotehnoloških procesa u proizvodnji aromatskih spojeva. Ovaj revijalni prikaz donosi nova dostignuća iz tog područja, osobito ističući mogućnosti primjene biljnih stanica i mikrobnih kultura te enzimske tehnologije u proizvodnji velikog broja aromatskih sastojaka hrane. Razmatra se i poboljšanje ekonomske isplativosti proizvodnje uzgojem mikroorganizama na čvrstoj podlozi.