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Flavor Compounds Produced by Fungi, Yeasts, and Bacteria

Carlos R. Soccol, Adriane B.P. Medeiros, Luciana P.S. Vandenberghe, and Adenise L. Woiciechowski

Divisão de Engenharia de Bioprocessos e Biotecnologia, Universidade Federal do Paraná, CEP 81531-970, Curitiba, PR, Brazil

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9.1 INTRODUCTION

Microorganisms play an important role in the generation of natural compounds, particularly in the field of food flavors. For a long time, plants were the sole sources of flavor compounds and most of them were isolated from essential oils. However, active compounds are present in low concentrations, which makes their isolation difficult. Another disadvantage of plants as a source of flavors is the dependence on factors that are difficult to control such as the weather and the risk of plant diseases. The production of flavor compounds by biotechnological methods has been an interesting alternative due to consumers' preference for natural ingredients. Microbial processes seem to be the most promising methods for the production of natural flavors.

9.2 MAIN TECHNOLOGIES

Many microorganisms are capable of synthesizing flavor compounds when growing on a culture media. They have the ability to perform conversions that would require multiple chemicals steps. Microorganisms are used to catalyze specific steps. They are also an economical source of enzymes, which can be utilized to enhance or alter the flavors of many food products (Kempler 1983). In this way, biotechnological processes involved in the production of flavor compounds can be divided into two groups: microbiological and enzymatic. Microbiological methods are subdivided into biosynthesis and biotransformation. The first is the production of chemical compounds by cells (fermentation or secondary metabolism). The second refers to the use of microbial cells in the specific modification of chemical structures (Welsh and others 1989).

In fermentation, the production of flavors starts from cheap and simple sources such as sugars and amino acids. The product is generated by the complex metabolism of the microorganism. When microorganisms are used in order to catalyze specific conversions of precursors and intermediates, the process is called biotransformation. Although fermentation requires C and N sources, a specific substrate is necessary for microbial transformation. The enzymatic catalysis precedes a simple and specific transformation of the substrate molecule. The substrate does not have to be "natural"; according to Schreier (1989) "non-natural" substrates can also be biotransformed.

It is important to distinguish research with the purpose of obtaining complex products with natural characteristics from those that try to obtain isolated molecules. The first consists in the experience of nature imitation and in developing a process with one or more microorganisms and enzymes. The second tries to obtain a higher yield of the characteristic components. The choice between them determines the methodology, which will be employed *in vivo* or *in vitro*, through biosynthesis or bioconversions (Delest 1995).

9.3 HOW TO OBTAIN FLAVORS

9.3.1 Flavor from Fermented Foods

The sensory properties of fermented foods are one of the key parameters in distinguishing these products from foods that have undergone undesirable spoilage. The organoleptic properties of fermented foods usually differ from those of the unfermented substrate and are dependant upon the biochemical activities of the associated microorganisms (Cook 1994).

Fermentation has been practiced for the production of food since ancient times. It has become an effective technology for the production of organic acids, flavor compounds, and other biologically important chemicals. New aroma and flavors includes acids, alcohols, carbonyl compounds, esters, and pyrazines.

The use of microorganisms in the production of food has been practiced for a long time to improve the sensory quality of the food. Products such as beer, wine, distilled beverages, bakery, vinegar, fermented vegetables, milk, soybean, and meat are preserved, modified, and flavored using microorganisms. The flavor compounds of traditionally fermented foods originate from a complex microflora that acts in the chemical precursors of a food matrix (Berger 1995).

Lactic acid and alcoholic fermentations are the two important processes responsible for fermented food flavors. However, in some cases, the flavor is formed by specific fermentations (Joshi and Pandey 1999). The creation of new fermented products can result in the development of novel flavors and textures.

9.3.1.1 Dairy Products. Cheese flavors find application in snacks, sauces, baked goods, and several other products. Yogurt and buttermilk flavors are also useful. The cheese flavor results from the action of microorganisms and enzymes on milk's proteins, fats, and carbohydrates. Numerous breakdown products are formed, among them, short-chain fatty acids, acetic and lactic acids, alcohols, aldehydes, ketones, esters, sulfur and nitrogen compounds (Sharpell 1985). Marilley and Casey (2004) have reported that the use of bacteria strains for cheese ripening with enhanced flavor production is promising. They also mentioned that the catabolism of amino acids is presumably the origin of some major flavor compounds.

The starter cultures used in dairy technology are mainly prokaryotes like *Lactococci*, *Lactobacilli*, *Leuconostocs*, *Bifidobacteria*, *Propionibacteria*, *Streptococci*, and *Brevibacterium linens*.

Yeasts, such as *Kluyveromyces*, *Debaromyces*, *Candida* or *Trichosporon* are present in many manufactured milk products. These microorganisms modify the sensory characteristics of the products by synthesizing or assimilating volatile nitrogen and sulfur compounds.

Several chemical reactions take place in the surface of ripened cheeses such as Camembert and Brie during maturation due to fungi growth. The Fungal mycelium of *Penicillium roqueforti* grows rapidly and the resulting products are used directly for flavoring foods with a blue cheese-type flavor.

9.3.1.2 Alcoholic Beverages. Flavor compounds are produced as byproducts of yeast metabolism during alcoholic fermentation. Many flavor compounds have been identified in alcoholic beverages. The main compounds are listed in Table 9.1.

During alcoholic fermentation, yeasts transform sugars (glucose, fructose, and sucrose) into ethanol and carbon dioxide by the Embden–Meyrhof–Parmas pathway. This is the main bioreaction, but not the only one and, at the same time, several secondary byproducts are formed. Higher alcohols, organic acids, and esters are the main flavor compounds.

Higher alcohols, which contain more than two carbons, are also called fusel alcohols. They constitute the major portion of the secondary products of yeast metabolism. They

TABLE 9.1 Compounds Produced by Yeasts During Alcoholic Fermentation.

Class	Compounds
Esters	Amylacetate, butyl acetate, ethyl acetate, ethyl butyrate, ethyl lactate, ethyl benzoate, ethyl hexanoate, ethyl guaiacol, ethyl-2-methyl butyrate, ethyl-3-methyl butyrate, ethyl octanoate, ethyl octenoate, ethyl decanoate, ethyl dodecanoate, diethyl succinate, 3-methyl propionate
Alcohols	Ethanol, 2-methyl butan-1-ol (amyl alcohol), methyl butan-1-ol (isoamyl alcohol), heptanol, hexan-1-ol, 2-phenyl ethanol, 2-methyl propanol, glycerol, 2,3-butanediol, n-propanol
Carbonyls	Decalactona, decan-2-one, acetaldehyde, butyraldehyde, hexanal, nonanal diacetyl benzaldehyde
Acids	Acetate, butyrate, lactate, malate, succinate, hexanoate, nonanoate, octanoate
Sulfur derivatives	Methionol, ethanetiol, methylthioacetate, dimethyl disulfide, ethyl methyl disulfide, diethyl disulfide, 3-methylthiopropyl acetate, 2-mercaptoethanol, *cis*- and *trans*-2-methylthiophanol
Phenolic compounds	Vinyl phenol, ethyl phenol, ethyl guaiacol, vinyl guaiacol

include n-propanol, isobutyl alcohol, 2-methyl butanol, amyl alcohol, isoamyl alcohol, and 2-phenyl ethanol. Isoamyl alcohol accounts for more than 50% of the total concentration of higher alcohols.

Esters at appropriate concentrations impart flowery and fruity flavors. They are formed by esterification of fatty acyl-CoA or of organic acid by alcohols. Esters are present in very low amounts, near their threshold level. However, ethyl acetate has been found in wine in high concentrations.

Beer. Acetaldehyde, the most important aldehyde in beer, is formed as a metabolic branch point in the pathway from carbohydrate to ethanol. Its level varies during fermentation and ageing of beers, reaching 2–20 mg/L. At concentrations of 20–25 mg/L, acetaldehyde causes "green" or "vegetable" flavor.

Diacetyl and pentane-2,3-dione (vicinal diketones) have a characteristic flavor described as "buttery", "honey" or "toffee-like". They have a very high off-flavor potential, dependent on the fermentation temperature. The threshold for diacetyl in lager-type beers is 0.10–0.14 mg/L. At levels above 1 mg/L, it becomes increasingly "cheese-like" and sharp.

Volatile acids are usually present in beer at concentrations of 20–150 mg/L. Butyric and iso-butyric acids in concentrations of 6 mg/L cause a "butyric" or "rancid" flavor. Valeric and iso-valeric acids cause an "old-hop" and "cheesy" flavor. Fatty acids with 6 to 12 carbon fatty acids give the characteristic flavor of "cheesy", "goaty", or "sweaty" (Smogrovicová and Dömény 1999).

Wine. The chemical composition of wine is determined by many factors, among them grape variety, geographical and viticultural conditions, microbial ecology of the grape, fermentation processes, and winemaking practices. Microorganisms affect the quality of the grape before harvest and during fermentation. They metabolize sugars and other components into ethanol, carbon dioxide, and hundreds of secondary products that contribute to the characteristic flavor of wine (Fleet 2003).

9.3.1.3 Bakery Products. Although *Candida* yeast has occasionally been used for baking and some *Saccharomyces carlsbergensis* strains have been patented for use as baker's yeast, pure strains of *Saccharomyces cerevisae* are almost universally employed.

9.3.1.4 Mushroom Flavors. The commercially important mushrooms belong to the orders *Ascomycetes* and *Basidiomycetes*. Truffles (*Tuber* sp.) and morels (*Morchella* sp.) represent the *Ascomycetes*. *Basidiomycetes* are represented by *Agaricus bisporus*, *A. bitorquis*, *Lentinus edodes* (Shiitake), *Volvariella volvacea*, *Pleurotus* sp., and *Flammulina velutipes*. The main chemical compound responsible for the mushroom flavor is 1-octen-3-ol, although several others, including glutamic acid and 5′-guanylic acid, can modify the flavor, giving each mushroom species its distinctive characteristic. There is interest in growing mushroom mycelium in submerged culture and then utilizing the dried mycelium as a flavor compound (Sharpell 1985).

9.3.2 Biosynthesis of Flavor Compounds

Biochemical reactions as well as several nonenzymatic reactions involving sugars, fatty acids, and amino acids give rise to flavor during fermentation. Several reports and reviews have been published on the production of volatile compounds by microorganisms (Janssens and others 1988, 1992; Berger 1995; Jiang 1995; Christen and others 1997; Bramorski and others 1998; Soares and others 2000; Medeiros and others 2001). Although several bacteria, yeasts, and fungi have been reported to produce flavor compounds, a few species of yeasts and fungi are often preferred. However, only a few of them find application in the food industries due to their GRAS (Generally Recognized As Safe) status.

Flavor compounds derived from microorganisms are often produced in low concentrations. These compounds have low thresholds and can be detected by chromatographic methods in parts per million (ppm, $\mu L/L$). The amount and type of compounds secreted by microorganisms depend on the strain, with its enzyme-specific action, chemical composition of the culture medium, pH and temperature control, age of inoculum, and water activity of the substrate.

Flavor compounds produced by *Trichoderma viride, Penicillium roqueforti*, and *Penicillium decumbens* have been detected during the phases of growth or sporulation, depending on the culture medium (Latrasse and others 1985).

Singhal and Kulkarni (1999) presented a schematic representation of the flavor compounds produced by microorganisms (Fig. 9.1). Some examples of flavor compounds produced by microorganisms are also indicated in Table 9.2.

9.3.2.1 Esters. Esters are a very important class of flavor compounds of fresh fruits and fermented foods, which are found in concentrations between 1 and 100 ppm (Janssens and others 1992). The production of the ester ethyl acetate by the yeast *Candida utilis* from glucose is observed when the yeast grows on a medium containing a specific initial ethanol concentration.

Esters of low molecular weight are responsible for fruity odors and consist of acids and their derived compounds such as acetates, propionates, and butyrates. Some examples are ethyl butyrate and isoamyl acetate, which are found in strawberry and banana (Macedo and Pastore 1997).

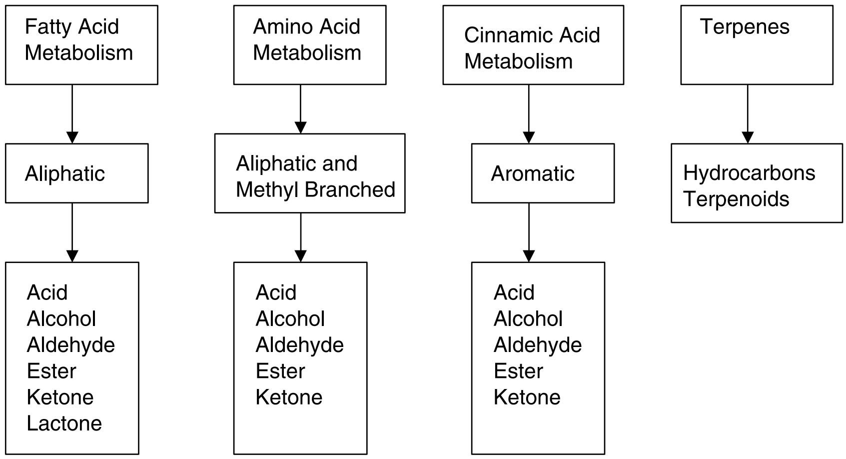

Figure 9.1 *Flavor compounds produced by microorganisms.*

The presence of esters such as ethyl acetate and butyric acetate in the culture medium can eventually describe a detoxification mechanism by which the microorganism avoids the accumulation of toxic compounds. The production of acetates occurs in order to detoxify the medium by converting acetic acid and high alcohols (Latrasse and others 1985).

TABLE 9.2 Microbial Production of Flavor Compounds.

Compounds	Microorganism
Diacetyl	*Saccharomyces lactis, Leuconostoc dextranicum*
Esters	
Geranyl acetate	*Ceratocystis virescens,C. variospora*
Ethyl butyrate and ethyl hexanoate	*Pseudomonas fragi*, *Streptococcus lactis*, *S. cremoris* *S. diacetylactis*, *L. casei*
Ethyl isovalerate	*Pseudomonas fragi*
2-Phenyl ethanol	*Erwinia carotovora*
Lactones	
α-Decalactone	*C. moniliformis*
t-Decalactone	*Sporobolomyces odorus*
s-Decalactone	*Saccharomyces cereviseae*, *Candida pseudotropicali*, *Sarcina lutea*
L-Menthol from menthyl esters	*Saccharomyces*, *Bacillus*, *Trichoderma*, *Candida*, *Rhizopus*
Pyrazynes	
2-Methoxy-3-isopropyl pyrazine	*Pseudomonas perolenes*, *Streptomyces*, *Streptococcus lactis*
Tetramethyl pyrazine	*B. subtilis*, *Corynebacterium glutanicum*
Terpenes	
Linalool	*Ceratocystic variospora*, *C. moniliformis*, *Phellinus igniarus*, *Kluveromyces lactis*, *Asocoidea, Lentinus lepidus*
Citronellol	*Ceratocystic variospora*, *C. moniliformis*, *K. lactis*, *Trametes odorate*
Geranyl acetate	*Ceratocystic virescens*
Citronemmyl acetate	*Ceratocystic coarulescens*

Source: Modified from Berger (1995) and Singhal and Kulkarni (1999).

Two metabolic pathways can be followed in the formation of esters: alcoholysis of acyl-CoA compounds and the direct esterification of an organic acid. Yeasts follow predominantly the first pathway, and filamentous fungi and bacteria prefer the second (Welsh and others 1989).

Some ramified amino acids are important precursors of flavor compounds and are related to fruit maturation. The initial reaction is called the enzymatic Strecker degradation. Several microorganisms including yeasts and bacteria such as *Streptococcus lactis* can modify the majority of the amino acid structures. Even if alcohols are related to fruit maturation, esters have a dominant role. Ethyl acetate comprises, with other compounds, banana flavor. 2-Methyl-ethyl-butyrate has a great impact on characteristic apple flavor (Fennema 1993).

Janssens and others (1987) found and quantified the fruity banana flavor produced by the yeast *Hansenula mrakii* and by the fungus *Geotrichum penicilliatum* in submerged fermentation using a synthetic medium. In the study using the yeast it was concluded that the fruity aroma occurred due to the biosynthesis of esters and alcohols. Seventeen compounds were identified in concentrations greater than 50 μL/L, including ethanol, ethyl acetate, isobutanol, ethyl propionate, isobutyl acetate, and isopenthyl acetate. Alcohols were formed in the exponential growth phase, but the esters were formed in the stationary phase. Ethyl acetate was the main product. In both studies, some precursors of fruity esters were added, such as vanillin, leucine, isoleucine, and phenylalanine. Thirty-three compounds were identified in concentrations greater than 50 μL/L: ethanol, ethyl acetate, ethyl propionate, and others. Ethyl acetate was produced in the highest concentrations (9924.1 μL/L).

Inoue and others (1994) reported the tolerance of *Hansenula mrakii* to ethyl acetate, which can be used as a sole carbon source. In this study, the esters formed during the production of sake by *Hansenula mrakii* and *Saccharomyces cerevisae*, were compared. Ethyl acetate, isobutyl acetate, and isoamyl acetate were preferably formed, determining the beverage quality. The formation of these compounds was catalyzed by the enzyme acetyl transferase from isoamylic acid and acetyl CoA.

Ethyl acetate, ethanol, acetic acid, and acetaldehyde were also produced by submerged fermentation and identified in the glucose metabolism of *Hansenula anomala*. In this case, the production of ethyl acetate was recognized as an aerobic process (Davies and others 1951).

Strains of *Ceratocystis* were also identified as ester producers. Lanza and others (1976) studied the production of acetates with different carbon (glucose, galactose, and glycerol) and nitrogen (urea and leucine) sources. They concluded that the type of flavor compounds produced depended on both sources (carbon and nitrogen), which is different for other microorganisms such as *Trichoderma viride*.

Collins and Morgan (1961) identified esters synthesized by different species of *Ceratocystis* (*C. moliniformis*, *C. major*, *C. coerulescens*, and *C. fimbriata*) during submerged fermentation in a dextrose potato medium. Ethyl acetate and ethanol were found in higher concentrations, except for *C. fimbriata*, which had isobutanol as the main compound. A strong banana flavor was detected when using dextrose and urea due to the presence of isoamyl acetate, which was also identified in leucine- or isoleucine-based media. For the combination galactose–urea, the main flavor was citric due to the formation of terpenes.

The yeast *Kluyveromyces marxianus* produced some compounds with characteristic fruity flavor, with ethyl acetate found in higher concentrations in solid-state fermentation of different agro-industrial residues such as cassava bagasse (Medeiros and others 2000, 2001).

Banana flavor has also been identified in some plants when microorganisms (*Erwinia caratovora* ssp atroseptica) infected them. Spinnler and Dijian (1991) identified the volatiles formed in a synthetic medium, similar to the ones in the infected plant. From 13 isolated microorganisms with capacity to produce esters and alcohols, several compounds were identified; including ethyl acetate, 2-methyl-1-propanol, and propyl acetate. In this study, different media were tested with different nitrogen and carbon sources. Better results were found with glucose, fructose, sucrose, and asparagine. The addition of leucine led to the production of isoamyl acetate, corresponding to the degradation of the amino acid following the Erlich route.

Beiju is made of naturally fermented cassava, with fruity characteristics. It was used by the indians in Maranhão, North of Brazil, in order to produce a typical alcoholic beverage, *tiquira*. The microbiological population was identified and quantified by Park and others (1982), with counts between 6×10^5 and 1.9×10^6 CFU, as being predominantly *Aspergillus niger* and *Pecylomyces* sp.

Yoshizawa and others (1988) identified some volatiles produced by a strain of *Neurospora*, isolated by Park and others (1982), in submerged fermentation. These include ethanol, ethyl acetate, isoamyl acetate, ethyl hexanoate, and acetoine.

9.3.2.2 Aldehydes. Aliphatic, aromatic, and terpenoid aldehydes are important contributors to the flavor of fermented dairy products. They are synthesized by microorganisms as intermediates in the formation of alcohols from keto acids. An example is the bioconversion of ethanol to acetaldehyde by *Candida utilis.*

Flavor production using immobilized lipase from the yeast *Candida cylindracea* in a nonaqueous system has been studied for producing a broad range of esters including ethyl butyrate, isoamyl acetate, and isobutyl acetate. Ethyl butyrate has a pineapple–banana flavor, which has a large market demand, and sells at a price of US$150/kg upwards. This process has shown a great stability of the enzyme (more than a month) if kept hydrated intermittently (Singhal and Kulkarni 1999).

9.3.2.3 Alcohols. Alcohols do not contribute as a flavor component unless present in high concentration. They are formed as a primary metabolite from microorganisms' activity or due to the reduction of a carbonyl. Fusel alcohols can be formed from either carbohydrate or amino-acid metabolism and are the predominant volatiles of all fermented beverages, in addition to ethanol.

Different alcohols can be found in the culture of yeasts such as ethanol, propanol, isobutanol, and phenyl ethyl alcohol. In filamentous fungi it is possible to find methyl-3-buthanol, butanol, isobutanol, pentanol, hexanol, octanol-3, and phenyl ethanol from the metabolism of amino acids such as leucine, valine, isoleucine, and phenylalanine (Welsh and others 1989).

9.3.2.4 Carbonyls. Among the ketones, odd-numbered 2-alkanones from five to eleven carbons, along with free fatty acids and 2-alkanols, determine the flavor of *Penicillium*-ripened cheese and have received much attention. Bacteria such as *Aureobasidium*, yeasts, and higher fungi produce 2-alkanones, but only *Penicillium* has been used industrially.

9.3.2.5 Terpenes. Terpenes are the most important natural components of essential oils to be used as flavors. Microorganisms are able not only to synthesize but also to degrade or transform terpenes. Fungi are the main microorganisms responsible for terpene production, but bacteria are capable of synthesizing a few volatile terpenoids, such as geosmin and cadin-4-ene-1-ol (Berger 1995).

The synthesis of monoterpenes by *Ceratocystis variospora* has been studied by Collins and Halim (1972). Numerous other microorganisms are able to synthesize monoterpenes, among them *Ceratocystis moniliformis*, *Kluyveromyces lactis*, *Sporobolomyces odorus*, *Trametes odorata* and *Trichoderma viridae.*

Microbial bioconversion of terpenes has been studied by several authors. Monoterpenoid compounds like citronellal, citral, limonene, and menthol (acetates) can be biotransformed in citronellol, geranic acid, carveol and 1-menthol, respectively.

Valencene is a sesquiterpenoid available from orange oil and has little commercial use. Some bacteria are capable of transforming valencene to nootkatone, a main flavoring component of grapefruit.

From the economic point of view, the development of biotechnological processes for the production of terpenes is not viable due to the low yields obtained and the abundance of vegetable sources available. The real meaning of these studies is the understanding of the steps of the catabolism of terpenes.

9.3.2.6 Lactones. Lactones are associated with fruity, coconut, buttery, sweet, or nutty flavors. *Trichoderma viridae*, a soil fungus, generates a characteristic coconut flavor due to the production of 6-pentyl-2-pyrone. The main component of peach flavor, 4-decalactone, can be synthesized by *Sporobolomyces odorus. Aspergillus niger* can transform β-ionone into a complex mixture resembling tobacco flavor. Lactones make a significant contribution to the flavor of several fermented foods like dairy products and alcoholic beverages.

Some microorganisms such as *Ceratocystic moniliformis*, *Trichoderma viride*, *Sporobolomyces odorus*, and some species of *Candida* have been reported as lactone producers. However, the production is not very significant and has low yields (mg/L), except for the *in situ* production of lactones from dairy products.

Lee and Chou (1994) verified that the addition of 3% castor oil to the culture medium raised the production of lactones by *Sporobolomyces odorus*, with a yield of 8.62 mg/L.

Among lactones, 6-pentyl-α-pyrone (6-PP) presents the most interesting flavor properties. It is a molecule with a strong coconut flavor and is also present in the aroma of peaches and nectarines. The production of 6-PP by *Trichoderma harzanium* with sugar cane bagasse by solid-state fermentation was studied by Sarhy-Bagnon (1999) as an alternative for the production by submerged fermentation, giving a six fold raise in concentration.

9.3.2.7 Pyrazines. Pyrazines are typical flavor components of heated foodstuffs. They give the roasted or nutty flavors characteristic of roasted nuts, coffee, and cocoa beans, and baked and meat products. Microwave foods need the addition of pyrazines because they do not develop a characteristic nonenzymatic browning flavor during cooking. *Bacillus subtilis* was the first organism found to produce pyrazine. Pyrazines were also identified in cultures of *Septoria nodorum* and *Aspergillus parasiticus*.

9.3.3 Enzymatic Technology

Enzymatic processes that are used to obtain flavors can be described by the hydrolysis of some compounds without microbial growth. The majority of the enzymes used in food processing are hydrolases, such as amylases, proteases, pectinases, cellulases, pentonases, invertase, and lactase. They are used, for example, in cheesemaking (lipases, proteases), wine and juice production (pectinases), lactose reduction (lactase). Immobilization techniques, such as gel inclusion, microcapsules entrapment, and covalent or adsorptive binding onto solid supports has improved technical aspects such as handling, recycling, and long-term stability. Microbial enzymes have become an integrated part of processes in the food industry, So it is natural to see their use for the generation of flavor compounds (Whitaker 1991).

Filamentous fungi are capable of producing enzymes that are used to hydrolyze plant cell wall and liberate its content. However, the enzymatic extraction needs a thermal treatment, which sometimes can destroy or change flavor compounds.

Pectinase, cellulase, and hemicellulase of *Aspergillus*, *Penicillium*, *Rhizopus*, and *Trichoderma* are enzymes more commonly used to increase extraction efficiency during fruit, vegetable, cereal, or juice processing (Armstrong and others 1989).

Lipases often show complex patterns after isoeletric focusing, but this heterogeneity is due to the varying degrees and positions of glycosilation of the protein core. Pre-pro-lipase and pro-peptides are now studied in detail by genetic engineering (Berger 1995).

Microbial hydrolases have been reported to improve the sensory quality of food by the synergistic action of mono-, oligo-, and poly-glucanases. Various carbohydrases have been purified and characterized, among them, β-glucosidase from *Aspergillus niger* and α-glucosidase, with maltase properties from banana pulp. Carbohydrases have contributed to the assessment of the identity and origin of plant products, to the understanding of changes during processing and maturation, and to the selection of flavor-rich cultivars. Stability and selectivity data will be decisive for sensory changes in a food and thus for the future application of new enzymes in food processing (Berger 1995). Smaller peptides and free amino acids, which are end products of various proteases, contribute to the non-volatile flavor fraction and act as precursors of volatiles.

Cheese treated with enzymes to enhance flavor, or a significant portion of the flavor profile, is considered to be enzyme-modified cheese (EMC). It provides the food manufacturer with a strong cheese note in a cost-effective, nutritious, and natural way (Moskowitz and Noelck 1987). Such EMCs are ideal in frozen cheese, because proteins from natural cheese tend to coagulate and produce a grainy texture, but the proteins in EMCs have been hydrolyzed to more soluble peptides and amino acids, overcoming these problems (Missel 1996).

EMC flavors available commercially include Cheddar, Muzzarela, Romano, Provolone, Feta, Parmesan, Blue, Gouda, Swiss, Emmental, Gruyere, Colby, and Brick. These cheese flavors have a wide range of applications in salad dressings, dips, soups, sauces, snacks, crisps, pasta products, cheese analogs, frozen foods, microwave meals, ready-made meals, canned foods, crackers, cake mixes, biscuits, quiches, gratins, cheese spreads, low-fat and no-fat cheese products, and cheese substitutes (Buhler 1996).

The basis of EMC technology is the use of specific enzymes acting at optimum conditions to produce typical cheese flavors from suitable substrates. These enzymes consist of proteinases, peptidases, lipases, and esterases. EMCs can be used in food recipes to fulfill several roles, for example, as the sole source of cheese flavor in a product, to intensify an existing cheesy taste, or to give a specific cheese character to a more bland-tasting cheese product (Anon 1993). They have approximately 15 to 30 times the flavor intensity of natural cheese and are available as pastes or spray-dried powders (Freund 1995). The production of EMC is an important industrial activity, which has grown due to a greater demand for convenience foods, together with the health-related concerns regarding the amounts of fat, cholesterol, and cholesterol-producing saturated fat in traditional dairy products. EMC has been included in no-fat and low-fat products, replacing the functional and flavor characteristics of fats previously derived from natural cheese (Anon 1993; Freund 1995). The addition of EMC creates the desired flavor without increasing fat content. It can be added at levels of 0.1% (w/w) and contribute less than 0.07% fat or 2.28 calories per 100 g. Most new applications are targeted at texture and provide rich mellow tones, pleasant flavor-enhancing effects, fatty mouth-feel, flavor masking, rounding-off of sharp spicy notes, and harmonization of other flavor ingredients (Buhler 1996).

REFERENCES

Anon. 1993. A savory assortment. Dairy Ingredients Int 58:30–31.

Armstrong DW, Gillies B, Yamazaki H. 1989. Natural flavors produced by biotechnological processing in flavour chemistry: Trends and Developments (ACS 388), 105–19.

Berger RG. 1995. Aroma biotechnology. Berlin: Springer-Verlag. 179 p.

Bramorski A, Christen P, Ramirez M, Soccol CR, Revah S. 1998. Production of volatile compounds by the fungus *Rhizopus oryzae* during solid-state cultivation on tropical agro-industrial substrates. Biotechnol Lett 20(4):359–62.

Buhler TJ. 1996. Natural dairy concentrates going beyond flavour. Food Technol Eur 3:56–8.

Christen P, Meza JC, Revah S. 1997. Fruity aroma production in solid-state fermentation by *Ceratocystis fimbriata*: influence of the substrate type and the presence of precursors. Mycolog Res 101(8):911–9.

Collins RP, Morgan ME. 1961. Identity of fruit-like aroma substances synthesized by endoconidial-forming fungi. Phytopathology 52:407–9.

Collins RP, Halim AF. 1972. Characterization of the major aroma constituent of the fungus *Trichoderma viride*. J Agric and Food Chem 20:437–8.

Cook PE. 1994. Fermented foods as biotechnological resources. Food Res Int 27:309–16.

Davies R, Falkiner EA, Wilkinson JF, Peel JL. 1951. Ester formation by yeasts – Ethyl acetate formation by *Hansenula* species. Biochem J 49:48–61.

Delest P. 1995. Natural flavours: Biotechnology limited or unlimited? In: Bioflavour'95, Dijon, France, INRA, Paris, Les colloques, n. 75.

Fennema OR. 1993. Quimica de los alimentos. Zaragoza: Editorial Acribia.

Fleet GH. 2003. Yeast interactions and wine flavour. Int J Food Microbiol 86:11–22.

Freund PR. 1995. Cheese flavours ripen into maturity. Int Food Ingred 5:35–7.

Inoue Y, Fukuda K, Wakai Y, Sudsai T, Kimura A. 1994. Ester formation by *Hansenula mrakii* IFO 0895: Contribution of esterease for iso-amyl acetate production in sake brewing. Lebensm Wiss U Technol 27:189–93.

Janssens L, De-Poorter HL, Vandamme EJ, Schamp NM. 1987. Production of flavours by the yeast *Hansenula mrakii*. Med Fac Landbouww Rijksuniv Gent 52:1907–13.

Janssens L, De-Poorter HL, Demey L, Vandamme EJ, Schamp NM. 1988. Fruity flavours by fermentation. Med Fac Landbouww Rijksuniv Gent 53(4b):2071–77.

Janssens L, De-Poorter HL, Vandamme EJ, Schamp NM. 1992. Production of flavours by microorganisms. Proc Biochem 27:195–215.

Jiang J. 1995. Changes in volatile composition of *Kluyveromyces lactis* broth during fermentation. In: Charalambous G, editor. Food flavors: Generation, analysis and process influence. p. 1073–86.

Joshi VK, Pandey, A. 1999. In: Joshi Vkand Pandey A, editors. Biotechnology: Food fermentation (microbiology, biochemistry and technology). Vol. I. New Delhi: Educational Publishers & Distributors, 521 p.

Kempler GM. 1983. Production of flavor compounds by microorganisms. Adv Appl Microbiol 29:29–51.

Lanza EO, Ko KHO, Palmer JK. 1976. Aroma production by cultures of ceratocystis moniliformis. J Agric Food Chem 24(6):1247–50.

Latrasse A, Degorcce-Dumas JR, Leveau JY. 1985. Production d'arômes par les microorganisms. Sciences des Aliments 5:1–26.

Lee SL, Chou CC. 1994. Growth and production of γ-decalactone and cis-6-dodecen-4-olide by *Sporobolomyces odorus* in the presence of fatty acids and oils. J Ferment and Bioeng 78(1):114–6.

Macedo GA, Pastore GM. 1997. Lipases microbianas na produção de ésteres formadores de aroma. Ciência e Tecnologia de Alimentos 17(2):115–9.

Marilley L, Casey MG. 2004. Flavours of cheese products: metabolic pathways, analytical tools and identification of producing strains. Int J Food Microbiol 90(2):139–59.

Medeiros ABP, Pandey A, Freitas RJS, Christen P, Soccol CR. 2000. Optimization of the production of aroma compounds by *Kluyveromyces marxianus* in solid-state fermentation using factorial design and response surface methodology. Biochem Eng J 6:33–39.

Medeiros ABP, Pandey A, Christen P, Freitas RJS, Fontoura PSG, Soccol CR. 2001. Aroma compounds produced by *Kluyveromyces marxianus* in solid state fermentation on packed bed column bioreactor. World J Microbio Biotechno 17:767–71.

Missel D. 1996. Selecting the right ingredients for adding the flavor cheese. Food Product Design 6:51–9.

Moskowitz GJ, Noelck SS. 1987. Enzyme modified cheese technology. J Dairy Sci 70:1761–9.

Park YK, Zenin CT, Ueda S, Martins CO, Neto JPM. 1982. Microflora in beiju and their biochemical characteristics. J Ferment Technol 60(1):1–4.

Sarhy-Bagnon, V. 1999. Production de 6-pentyl-a-pyrone par *Trichoderma harzanium* cultivé sur support solide. Doctorat Thesis, Université Montpellier II.

Schreier P. 1989. Aspects of biotechnological production of food flavors. Food Rev Int 5(3): 289–315.

Sharpell Jr FH. 1985. The principles, applications & regulations of biotechnology in industry, agriculture and medicine. In: Moo-Young M, editor, Comprehensive Biotechnology. n. 3. Canada, Toronto: Pergamon Press.

Singhal RS, Kulkarni PR. 1999. Production of food additives by fermentation. In: Joshi VK, Pandey, A. editors. Biotechnology: Food fermentation (microbiology, biochemistry and technology). Vol. II. New Delhi: Educational Publishers & Distributors, 1372 p.

Smogrovicová D, Dömény Z. 1999. Beer volatile by-product formation at different fermentation temperature using immobilized yeasts. Proc Biochem 34:785–94.

Soares M, Christen P, Pandey A, Soccol CR. 2000. Fruity flavour production by *Ceratocystis fimbriata* grown on coffee husk in solid-state fermentation. Process Biochemi 35:857–61.

Spinnler HE, Djian A. 1991. Bioconversion of amino acids into flavouring alcohols and esters by *Erwinia carotovora* subsp. *Atroseptica*. Appl Microbiol Biotechnol 35:264–29.

Welsh FW, Murray WD, Willians RE. 1989. Microbiological and enzymatic production of flavor and fragrance chemicals. Criti Rev in Biotechnol 9(2):105–69.

Whitaker JR. 1991. Enzymes: Monitors of food stability and quality. Trends Food Sci Technol 2:94–7.

Yoshizawa K, Yamauchi H, Hasuo T, Akita O, Hara S. 1988. Production of a fruity odor by *Neurospora* sp. Agricult Biolog Chem 52(8):2129–30.

10

Flavor Production by Solid and Liquid Fermentation

Carlos R. Soccol, Adriane B.P. Medeiros, Luciana P.S. Vandenberghe, and Adenise L. Woiciechowski

Divisão de Engenharia de Bioprocessos e Biotecnologia, Universidade Federal do Paraná, CEP 81531-970, Curitiba, PR, Brazil

10.1 INTRODUCTION

Microbial fermentation is a promising biotechnological technique for the production of natural flavors. Although many biotechnological processes have been reported, most have not yet been applied in industrial production. The major reason for this is the low yield. Microbial flavors are often present in low concentrations in fermentation broths,

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SPECIFIC GRAVITY OF SOLIDS

Measures

Specific gravity by weight of known volume.

Uses

Measure amount of air incorporated in products such as whipped cream, egg white foams, creamed shortening, and cake batters.

Procedure

1. Weigh a dry container to the nearest gram.
2. Fill container with cooled, boiled deionized water at room temperature. Complete fill on balance; judge at eye level. Weigh to nearest gram.
3. Fill dry cup with test material. Do not pack. Remove excess with spatula. Wipe outside of container. Weigh to nearest gram.
4. Calculate the specific gravity as follows:

$$\text{specific gravity} = \frac{\text{weight filled container} - \text{weight container}}{\text{volume container}}$$

where volume container = (weight container + water) – weight container. Since specific gravity is the density of a substance relative to water it has no units.

SPECTROPHOTOMETER

Measures

The absorption of light at a particular wavelength by the sample.

Uses

Qualitative identification and quantitative determination of colored substances.

Procedure

1. Turn on instrument by rotating the left knob clockwise and allow to warm up for 15 to 30 min (see Figure 20.16). Turn wavelength dial to appropriate setting.
2. Zero instrument with cuvette chamber empty and lid closed by adjusting left knob until needle is at 0% transmittance. Insert cuvette containing reagent or tissue blank and adjust right knob until needle reads 100% transmittance. Each time the wavelength is changed, zero the instrument again.

Figure 20.16 Spectrophotometer.