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FLAVOR CHEMISTRY and TECHNOLOGY

GARY REINECCIUS

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9 Flavoring Materials Made by Processing

9.1 INTRODUCTION

The term *processed flavor* is used in different contexts to describe: (a) products where the natural raw materials lack a characteristic flavor profile and the desired aromatic profile is achieved only by deliberate processing (e.g., coffee); (b) flavorings created as a result of Maillard and other related reactions between amino acids and sugars (e.g., meat-like flavors); (c) flavorings resulting from controlled enzymatic reactions (e.g., enzyme-modified dairy products); (d) products made by fermentation (e.g., wines, vinegar); and (e) products of thermal reactions of lipids (e.g. French fry flavor).

Some of these flavorings are produced as a result of a combination of these processing methods (e.g., coffee and cocoa, which involve fermentation and roasting; tea, which may or may not be fermented before roasting; vanilla, which must be fermented and cured; and thermally processed enzymatically modified dairy products). Some of these products (or reactions leading to products) have already been discussed in Chapter 5, but there will be a greater emphasis in this chapter in producing flavorings rather than just discussing the chemistry involved.

9.2 NATURAL PRODUCTS MADE BY ROASTING: COCOA/CHOCOLATE

The production of cocoa and chocolate from cocoa beans involves three prime processes: (a) fermentation, (b) drying, and (c) roasting, each of which has a significant effect upon the final flavor profile. The chemistry involved is complex [1–3] and the quantitative mix of aromatic constituents resulting from processing is determined by the precise conditions employed. Processing may be summarized as follows:

1. Fermentation facilitates separation of beans from the surrounding pulp and shell, inactivates the seeds, initiates the aromatic precursors and color development, and significantly reduces bitterness. During this stage the sucrose and proteinaceous constituents are partially hydrolyzed, any polyphenolic compounds are oxidized and glucose is converted into alcohol and then oxidized to acetic and lactic acids. Beans that have not been fermented do not develop a chocolate flavor when roasted [4].
2. Drying removes moisture to about 15%.
3. Roasting develops the desired flavor profile and completes the color changes, further reduces the moisture content to about 5%, conditions the

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shell facilitating its removal, and makes the nib more friable. During roasting a portion of the initial fermentation products are converted by Maillard reactions into a complex mixture of volatile carbonyls, pyrazines, etc., which characterize the aroma and flavor of the final chocolate [5–7].

The temperature conditions encountered during the roasting process depend on the type of roaster employed, but they usually lie within the range 115 to 140°C. The precise conditions are still one of skill and judgment based on knowledge of the raw materials used and the ultimate product being made.

9.2.1 Production of Cocoa Powder

Having roasted the beans, it is necessary first to separate the shell from the kernel by passing it through rollers and then separating the broken shell and the cocoa nibs by winnowing, using graded screens and air elution. Final separation is carried out to ensure that the nibs are completely free from shell (shell is limited to 1.75% by law) prior to the next stage of processing.

Cocoa powder is manufactured by grinding the press cake after partial removal of cocoa butter. This product demands high roasted beans to give the end product a better flavor and darker color. Subjecting the separated nibs to a pressure of 7,000 lb/in^2 leaves a residual 20 to 28% cocoa butter in the press cake. On leaving the press, the cake is broken to form small pieces of about 1 inch size, which are then subjected to further milling under carefully regulated temperature conditions and cooled rapidly to 20 to 23°C immediately on discharge, to ensure a properly tempered cocoa butter content, the correct particle size, and an acceptable color.

Cocoa powder contains about 24% fat, 18% nitrogenous matter, 5.5% fiber, 10.5% starch, and about 4% moisture. When permitted, commercial products may contain additives such as sucrose esters, tripolyphosphate, disodium sulfosuccinate, or alginates in order to improve "wettability" as well as vanillin and other flavorings to give them a more rounded profile.

9.2.2 The Dutch Process

Dutching, or alkalizing, is carried out to modify the color and flavor of cocoa. Depending on the degree of alkalization, the color becomes darker and the flavor milder and much less harsh. This process involves the addition of alkali to the milled chocolate liquor accompanied by efficient mixing and the removal of water by heating.

9.2.3 Chocolate

Chocolate is a suspension of cocoa particles and sugar held in cocoa butter, the manufacture of which entails four unit operations:

1. Mixing is done with the correct proportions of sugar, cocoa butter, and cocoa liquor in a special plant known as a melangeur. This consists of two heavy rollers mounted over a heated rotating steel or granite bed, although other designs for continuous automatic operation are widely used

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so that mixing and flavor development are optimized. The mixing operation is carried out at 50 to 60°C and usually takes 20 to 25 min, but in an automatic plant the dwell time may be only about 5 min. The aim of this stage is to reduce the particle size of the sugar and promote the loss of unwanted volatile acids. The product is a soft, plastic, pliable, non-flowing mass.

2. Refining is a size reduction process. This is carried out in a mill fitted with multiple, water cooled rollers [3–5]. Chocolate is usually refined to a particle size of 50–114 μm of which less than 10% are smaller than 5 μm and not more than 20% are larger than 22 μm. It is the distribution of particle sizes that determines the eating quality of the resulting chocolate [8].
3. Conching is necessary to ensure full flavor development by the elimination of the remaining volatile acids; it also removes moisture, smoothes angular sugar crystals, modifies the viscosity and alters the color of the product. The specially designed equipment produces efficient agitation and aeration to ensure good mixing and exposure of the product to air over a period of between 1 and 5 days at a controlled temperature below 100°C, depending on the type of chocolate being made. During conching, further oxidative changes occur together with some caramelization.
4. Tempering is necessary to induce crystallization of the cocoa butter in a stable form in the fluid chocolate mass. Cocoa butter can crystallize in six forms: I (γ, melts at 17°C and is very unstable), II (α, melts at 21 to 24°C), III and IV (β′, melts at 25 to 29°C), V (β, melts at 34 to 35°C and is the preferred form), and VI (associated with chocolate bloom) [9,10].

The use of cocoa and chocolate products as flavoring materials is described by Minifie [9] and Beckett [10]. Key volatiles responsible for the characterizing aroma of chocolate have been reported by Schnermann and Schieberle [11] and Schieberle and Pfnuer [12] (briefly summarized in section 8.2.2.7.2 Cocoa/Chocolate Flavor).

9.3 PROCESS FLAVORS: MEAT-LIKE FLAVORS

The Maillard reaction can produce very many volatile compounds having strong and distinctive odors, depending on the reactants and the reaction conditions [13]. The classical Maillard reaction and the closely associated Strecker degradation have already been discussed in Chapter 5. Although these two reactions are only a part of the total chemical changes occurring during the heat treatment of foods, they generate more characteristic flavoring effects in processed foods than any other reaction.

The reproduction of meat-like flavors through process chemistry has been a primary target of the flavor industry for many years. Meats are expensive, and thus there is a strong financial incentive to develop substitute flavorings. The chemistry of raw and cooked meat flavor has been the subject of considerable research over the past 30 years, and this has provided the flavor industry with invaluable data for the recreation of process meat-like flavorings [14–18].

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9.3.1 The Evolution of Process Meat-Like Flavorings

The development of meat-like flavorings has been an evolutionary process [19]. Early meat-like flavorings would probably more correctly be called meat extenders and were largely made from spice blends. The manufacturer would use spices normally associated with specific meats and sell them as meat enhancers or extenders. In the U.S. culture, one can envision sage being associated with pork, turmeric and celery with chicken, and onion and black pepper with beef. The use of these spice combinations did little to enhance the true meat flavor, but at least there was a flavor. Meat extracts have found some use in the industry and still do today. These byproducts of the meat processing industry (e.g., corned beef) provide little desirable flavor but contribute to a label statement of meat being present.

The beginning of thermal processes as a means to produce meat-like flavorings was the introduction of hydrolyzed vegetable proteins (HVPs). These products are thermally processed and bring both a characteristic flavor of their own (primarily from processing) and an abundance of flavor enhancers, particularly monosodium glutamate (MSG). Recent concern over the presence of monochloropropanol and dichloropropanol (suspected carcinogens) in HVPs has resulted in a new generation of HVPs produced through enzymatic action as opposed to heat and acid [20,21]. However, these new products do not offer the same flavor profile as the traditional products and thus are not as desirable.

The discovery and commercialization of savory flavor enhancers were a boon to these flavorings. The contribution of MSG and the 5′ nucleotides cannot be overestimated. While the consumer often states that these ingredients are not desired, they are intimately associated with the recognized meat-like flavorings and are indispensable. The final development in these flavorings has been the advent of top-noting, i.e., the addition of synthetic chemicals that add potency or specific sensory nuances to the base flavor. Aroma chemicals such as 2-methyl-3-furanthiol fortify the characteristic flavor (e.g., beef in this case) of the meat-like flavoring and thereby afford greater flavor potency, which is generally lacking in this flavoring group. Other chemicals may be added to provide a given sensory nuance such as a rare, bloody, or roasted note to the base flavor.

The advent of gas chromatography and interest by the academic community in flavor chemistry in the 1960s offered promise that the aroma of meat could be reproduced synthetically. It was proposed that if one could identify all of the aroma components of meat, the aroma of that meat could be reconstituted from pure chemicals to yield a quality, formulated meat flavoring. This promise has never been fully realized for several reasons. One was that meat aroma proved to be extremely complex, being made up of well over 800 aroma chemicals. A large number of these aroma compounds either are not commercially available or cannot be added to foods for legal reasons (i.e., a lack of toxicological data). Simply the challenge of reformulating 800+ aroma chemicals into a flavoring in a processing environment is daunting. These limitations resulted in efforts to determine which chemicals were truly *required* to provide a characteristic meat aroma. It has been proposed that 20 to 30 chemicals may be adequate to reproduce most food flavors so the question was which 20 to 30 chemicals? If these chemicals are available and permitted to be

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used in foods, then perhaps we can formulate synthetic meat-flavors. This line of research was pursued in the 1980s and '90s largely by Prof. Grosch and his research group in Garching. At this time, we have solid data on the chemicals needed to recreate meat aroma [22–32]. The primary hurdle today is that many of these chemicals are not available as natural compounds, and perhaps this blend of volatiles still lacks elements of carrying all of the characteristic meat flavor, i.e., aroma and taste.

The inability of the flavor industry to offer natural meat flavorings via chemical recombination spawned considerable research on reaction systems (meat flavor precursor systems). In the 1960s and '70s, a host of patents were issued outlining reaction formulations and processing conditions that created meat-like flavorings [19]. These flavorings did a reasonable job of providing both the taste and aroma of meats with the added benefit of carrying a natural label (provided the ingredients were natural and process conditions within approved guidelines). Much of this work was based on studies that pointed to the presence and consumption of certain flavor precursors in meats (primarily amino acids and reducing sugars) during cooking. It was assumed that a given flavor, e.g., roasted beef, could be formed in a reaction system from the same primary reactants and reaction conditions as exist in the product to be imitated, e.g., beef. This fundamental research, as well as a large helping of trial and error, has lead to an understanding of how to create meat-like flavorings through process chemistry. Processes for creating these flavorings will be described in the following sections. International Organization of the Flavor Industry (IOFI) guidelines for the creation of these flavorings are presented in Table 9.1.

9.3.2 The Creation of Process Flavorings

9.3.2.1 Reaction System Composition

The base ingredients used in process flavors generally include: (a) a protein nitrogen source, (b) a carbohydrate, (c) a fat or fatty acid, (d) water, (e) a pH regulator(s), and (f) various flavor enhancers. Depending upon the flavor and the creator, numerous other ingredients may be added to provide a given sensory note, e.g., smoke, or spice.

9.2.2.1.1 Sulfur Sources

If one is creating a meat-like flavor, a sulfur source is required in the reaction mixture. This sulfur source is most commonly cysteine and/or cystine (free acids or hydrochlorides). However, hydrogen sulfide has been used as a replacement (since the sulfur amino acid is typically considered a generator of hydrogen sulfide), but hydrogen sulfide is both corrosive and toxic in the workplace, and thus manufacturers prefer to work with the amino acids. Thiamin is an excellent source of hydrogen sulfide and will form various meat-like flavors by heating it alone (in water) at selected pHs, times, and temperatures. The aroma of heated thiamin initially is egg-like (hydrogen sulfide), but then it becomes more chicken-like and finally beef-like. While thiamin is an excellent precursor for the creation of meat-like flavorings, its cost, natural status, and vitamin activity (legal considerations) all are negative factors. Methionine is used to provide vegetable notes (potato due to its thermal degradation

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TABLE 9.1
International Organization of the Flavor Industry (I.O.F.I.) Guidelines for the Production and Labeling of Process Flavorings

Process flavorings are produced by heating raw materials which are foodstuffs or constituents of foodstuffs in similarity with the cooking of food.

The most practicable way to characterize process flavorings is by their starting materials and processing conditions, since the resulting composition is extremely complex, being analogous to the composition or cooked foods. They are produced every day by the housewife in the kitchen, by the food industry during food processing and by the flavor industry.

The member associations of I.O.F.I. have adopted the following Guidelines in order to assure the food industry and the ultimate consumer of food of the quality, safety and compliance with legislation of process flavorings.

1. *Scope*
   1.1. These Guidelines deal with thermal process flavorings; they do not apply to foods, flavoring extracts, defined flavoring substances or mixtures of flavoring substances and flavor enhancers.
   1.2. These Guidelines define those raw materials and process conditions which are similar to the cooking of food and which give process flavorings that are admissible without further evaluation.
2. *Definition*
   A thermal process flavoring is a product prepared for its flavoring properties by heating food ingredients and/or ingredients which are permitted for use in foodstuffs or in process flavorings.
3. *Basic Standards of Good Manufacturing Practice*
   The chapter 3 of the Code of Practice for the Flavor Industry is also applicable to process flavorings.
4. *Production of Process Flavorings*
   Process flavorings shall comply with national legislation and shall also conform to the following:
   4.1. Raw materials for process flavorings. Raw materials for process flavorings shall consist of one or more of the following:
      4.1.1. A protein nitrogen source:
         - Protein nitrogen containing foods (meat, poultry, eggs, dairy products, fish, seafood, cereals, vegetable products, fruits, yeasts) and their extracts
         - Hydrolysis products of the above, autolysed yeasts, peptides, amino acids and/or their salts.
      4.1.2. A carbohydrate source:
         - Foods containing carbohydrates (cereals, vegetable products, and fruits) and their extracts
         - Mono-, di- and polysaccharides (sugars, dextrins, starches, and edible gums)
      4.1.3. A fat or fatly acid source: foods containing fats and oils
         - Edible fats and oils from animal, marine, or vegetable origin
         - Hydrogenated, transesterified, and/or fractionated fats and oils
         - Hydrolysis products of the above.
      4.1.4. Materials listed in Table 9.2.
   4.2. Ingredients of process flavorings
      4.2.1. Natural flavorings, natural and nature identical flavoring substances and flavor enhancers as defined in the I.O.F.I. Code of Practice for the flavor industry.
      4.2.2. Process flavor adjuncts. Suitable carriers, antioxidants, preserving agents, emulsifiers, stabilizers and anticaking agents listed in the lists of flavor adjuncts in Annex II of the I.O.F.I. Code of Practice for the flavor industry.

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TABLE 9.1 (continued)
International Organization of the Flavor Industry (I.O.F.I.) Guidelines for the Production and Labeling of Process Flavorings

4.3. Preparation of process flavorings. Process flavorings are prepared by processing together raw materials listed under 4.1 and 4.1.2 with the possible addition of one or more or the materials listed under 4.1.3 and 4.1.4.

4.3.1. The product temperature during processing shall not exceed 180°C.

4.3.2. The processing time shall not exceed ¼ hour at 180°C with correspondingly longer times at lower temperatures.

4.3.3. The pH during processing shall not exceed 8.

4.3.4. Flavorings, flavoring substances and flavor enhancers (4.2.1) and process flavor adjuncts (4.2.2) shall only be added after processing is completed.

4.4. General requirements for process flavorings.

4.4.1. Process flavorings shall be prepared in accordance with the General Principles of Food Hygiene (CAC/Vol A-Ed. 2 (1985) recommended by the Codex Alimentarius Commission.

4.4.2. The restrictive list of natural and nature-identical flavoring substances of the I.O.F.I. Code of Practice for the Flavor Industry applies also to process flavorings.

5. *Labeling*

The labeling of process flavorings shall comply with national legislation.

5.1. Adequate information shall be provided to enable the food manufacturer to observe the legal requirements for his products.

5.2. The name and address of the manufacturer or the distributor of the process flavoring shall be shown on the label.

5.3. Process flavor adjuncts have to be declared only in case they have a technological function in the finished food.

to methional) to the flavoring. The patent literature abounds with suggestions for other sulfur sources, which include a host of plant extracts (e.g., extracts from the Allium family) as well as pure chemicals (e.g., methanethiol, ethanethiol, and dimethylsulfide). However, the amino acids mentioned earlier are the workhorses of this group of flavorings.

9.3.2.1.2 Reducing Sugars

The second critical component of meat-like flavorings is a reducing sugar. While amino acid choice strongly influences the sensory character of the final flavoring, carbohydrate choice has less influence on character. Reducing sugars cannot arbitrarily be interchanged in a reaction mixture, but this choice has a more subtle influence on flavor character.

Glucose is the most common sugar used for this purpose. Glucose is cheap and reasonably reactive. Arabinose, xylose, fructose, and lactose also find application in various meat-like flavorings. Of these latter reducing sugars, the use of xylose has increased in recent years. Xylose, being a pentose, is more reactive in the Maillard reaction and thus shorter process times are possible. The cost of xylose has until recently prohibited its use in process flavorings.

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TABLE 9.2
Materials Used in Procession

Herbs, spices, and their extracts
Water
Thaimine and its hydrochloric acid salt
Ascorbic, citric, lactic, fumaric, succinic, and tartaric acids
The sodium, potassium, calcium, magnesium, and aluminum salts of the above acids
Guanylic acid and inosinic acid and its sodium, potassium and calcium salts
Inositol
Sodium, potassium, and ammonium sulfides and hydrosulfides, and polysulfides
Lecithin
Acids, bases and salts as pH regulators
  Acetic, hydrochloric, phosphoric, and sulphuric acids
  Sodium, potassium, calcium, and ammonium hydroxide
  The salts of the above acids and bases
Polymethylsiloxane as an antifoaming agent (participating in the process)

Source: From Manley, C.H., Flavor Interactions Workshop, IFT sponsored, Orlando, FL, 2003. With permission.

Ribose, ribose-5-phosphate, hydrolyzed polysaccharides, yeast, and yeast autolysates also appear in the patent literature as carbohydrate sources for these flavorings. There are no economical sources of ribose or ribose-5-phosphate. Yeast and yeast autolysates both will contribute some free carbohydrate.

Similar to the amino acids, the carbohydrate may be replaced by a pure chemical such as propanal or diacetyl. The Maillard reaction is broadly the reaction of an amine with a carbonyl, and thus the carbonyl does not have to be a reducing sugar.

9.3.2.1.3 Acids

Acids are typically used to adjust the pH of the reaction system. Meat-like flavorings are generally thermally processed at pHs around 5.2, similar to the pH of meat. Phosphoric acid is most commonly used since it is known to be a catalyst of the Maillard reaction. Other inorganic acids also may be used such as hydrochloric or sulfuric acids. Numerous organic acids appear in the patent literature. These acids typically contribute a flavor of their own and may be used to not only produce a given pH but also add a desired sensory note. Citric, lactic, acetic, propionic, malic, succinic, and tartaric acids appear in the patent literature.

9.3.2.1.4 Lipids

Some of the earlier research on meat flavors demonstrated that the common meaty characteristic of cooked meats can be reproduced by heating the water soluble components of the meats [33–35,16]. However, the differentiation amongst species was found when the lipid portion of the meat was heated. This has resulted in most meat-like flavorings using a similar base meat reaction system but then using added lipid to provide the unique species character of the meat, lamb or fish, for example.

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The lipid is typically fat trimmings from the animal whose flavor is to be reproduced. While the industry is now able to add synthetic chemicals to create much of this species differentiation without using lipid in the reaction system, the chemicals are not natural, and the product must carry an artificial label.

The addition of lipids (as animal byproducts) to the reaction system is problematic. The issues resulting from this addition include:

1. Lipids are insoluble in the reaction system, so the flavoring either has a fat cap (not homogeneous) or has to include emulsifiers (label issue)
2. The lipids result in a cloudy product upon final use — clear stocks cannot be prepared
3. If a dry flavor form is desired, lipids make drying more difficult
4. The final product may have an oil slick (appearance)
5. Religious reasons (Kosher, Halal)
6. Fear of animal products (mad cow disease)
7. The lipids must be stabilized against oxidation (label issue)
8. The use of animal byproducts requires that the flavor company has a USDA compliant manufacturing facility including inspection

Probably the largest barrier to most companies using lipids (as animal byproducts) is this latter requirement. A flavor company uses very few materials that support microbial growth and, in fact, resembles a chemical company more than a food company. Thus, the need to operate the equivalent of a meat processing facility is outside their normal operating environment.

Some of the listed issues are irrelevant when nonanimal lipid sources are used. Thus one finds numerous patents proposing that plant oils or specific fatty acids may be used to create species character (the author is not aware of there being much success in this respect).

9.3.2.1.5 Solvents

The patent literature lists several solvents for use in the creation of meat-like flavorings. They include water, alcohol, propylene glycol, and glycerol. For meat-like flavorings, water is by far the most commonly used solvent. The only reason to consider a different solvent is to alter the water activity of the reaction system. For meat-like flavorings, this rationale is not compelling because meats have very high water activities, which must be duplicated in the reaction system. In the case of creating a chocolate or coffee process flavoring, nonaqueous solvents are commonly used since the cocoa and coffee beans are dry when roasted, and thus a low water activity must be obtained in the reaction system to duplicate this condition.

9.3.2.1.6 Hydrolyzed Vegetable Proteins (HVP), Savory Enhancers (MSG and 5′-Nucleotides), and Autolyzed Yeast Extracts (AYE)

These common components of meat-like flavorings are nearly universally used in the industry [19]. While there has been and continues to be a negative consumer attitude towards the use of MSG and HVP, their use continues since they are essential to the sensory character of meat-like flavorings. (MSG is associated with the Chinese

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Restaurant Syndrome. HVPs are not desired because they may contain the earlier noted carcinogens [Section 9.3.1] and they are abundant sources of MSG). The historical, abundant use of the HVPs, MSG, and AYEs in these flavorings has resulted in their characteristic flavor being recognized as an integral part of the flavoring despite real meat having only subtle touches of these components. Some companies have chosen to offer meat-like flavorings without these components, but they have very little success in the marketplace. Since each of these ingredients will be discussed later in this chapter or in Chapter 11 (Flavor Potentiators), there will be no additional discussion here.

9.3.2.1.7 Miscellaneous Components

In addition to the components discussed above, numerous minor (in quantity) components may be added to the reaction system after thermal treatment. They include:

Salt — There may be more than enough salt in the flavoring already due to the use of HVPs or neutralization of the reaction mixture (the reaction mixture is normally neutralized after thermal treatment). However, in some applications, additional salt may be needed.

Preservatives — Preservatives (e.g., chelators or antioxidants) may be added after processing to inhibit the oxidation of lipids used in the reaction mixture.

Thickeners — Thickeners (e.g., gums or starches) may be added to the reacted mixture to stabilize the system against physical separation. The reaction mixture may contain various insoluble components (not only lipids) that will separate during cooling and storage. Increasing the viscosity will help provide a homogeneous product.

Surfactants — Surfactants (e.g., lecithins or monoglycerides) may be added after processing to emulsify the lipids into the reaction system. This again insures a homogeneous flavoring.

Flavorants — Numerous flavorants may be added after processing. They may be complete flavorings such as a smoke flavoring, various spice or herb essential oils, or oleoresins. The addition of top notes also may be put into this category. Top noting refers to the addition of individual pure chemicals that strengthen or modify the character of the process flavor. Top noting has become very common (as was mentioned earlier) and is a current area of significant research.

Colorants — There is little rationale to add colorants (e.g., caramel color) to a meat-like flavoring other than for the convenience of the final user. The colorant would be added to color the final product, thus the choice of the colorant and its usage level would be customer product dependent.

Drying agents — Drying agents (e.g., low DE maltodextrins) may be added to the processed reaction mixture to facilitate drying (provide a dry flavor form). Processed reaction mixtures are very hygroscopic and become sticky at elevated temperatures, and thus are difficult to dry without the addition of some drying aid. Low DE maltodextrins are commonly used for this purpose since they are cheap, bland, and dry well. Pan (or tray) drying may be accomplished with minimal addition of a drying aid. However, if one

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desires a spray dried product, it may be necessary to use nearly a 1:1 (solids basis) ratio of maltodextrin to reaction mixture solids. This results in a significant dilution of the flavoring. One of the common criticisms of this flavor category is that they lack flavor strength. Dilution of the flavoring to facilitate drying exacerbates this weakness.

Free flowing agents — As noted above, reaction products are very hygroscopic. This property means that they will very readily cake on storage unless some protection against moisture uptake is provided. Caking is minimized by adding drying aids to increase the glass transition temperature of the mixture, using a good vapor barrier packaging, and/or adding silicates. Adding drying aids dilute the product (as noted earlier), and thus this is not necessarily the desired choice. Applying packaging with high water vapor barrier properties adds cost and has minimal value once the package is opened. The use of free flowing agents is the common approach. Silicates will absorb considerable moisture thereby reducing the need for expensive packaging. It must be remembered, however, that silicates will not stop caking but only prolong the time to caking. The process flavor will cake at the same water activity with or without silicate, but the competitive absorption of water by the silicates simply prolongs the time until this water activity is reached.

9.3.2.2 Reaction Conditions

Different blends of reactants may be heated under a wide range of conditions (including solids content and pH) to create a wide range of flavorings. The IOFI and Flavor Extract Manufacturers Association (FEMA) have established guidelines for the thermal processing of such products as not exceeding 15 min at 180°C or proportionately longer at lower temperatures. More recently, additional regulations have come into being that set minimum processing conditions. As a result of concern that some food processors may be adding water binding ingredients (e.g., proteins) to meat-products under the guise of natural flavorings, it is now required that these flavorings be processed minimally at 100°C for 15 min.

The time and temperature of heating strongly influence the flavor character of the process flavoring. It is generally acknowledged that heating temperatures of ca. 100°C yield cooked or stewed meat flavors and temperatures of ca. 120°C produce roasted meat flavors. Heating may be done in an open pan (which allows volatiles to escape) if the system is heated at temperatures equal to or below boiling, or in a closed vessel (maintains volatiles in the system) if desired, or higher temperatures are used. Capturing volatiles or allowing them to escape influences the flavor character. In some processes, the manufacturer may choose to heat a part of the formulation under one set of conditions and then add additional ingredients and further heat.

While the pH during heating is generally about 5.2, IOFI and FEMA guidelines state that pH should be between 3 and 8. After heating, the pH is generally adjusted to neutrality. Examples of meat-like flavoring formulations and processing were presented by Manley [36] and are summarized in Table 9.3.

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TABLE 9.3
Examples of Process Flavor Formulations and Processing

Roast Beef Type Flavor	
HVP	Sodium guanylate
Malic acid	L-methionine
Xylose	Water
Reflux at 100°C for 2.5 h	

Chicken Type Flavor	
L-cysteine HCl	Glycine HCl
Glucose	L-arabinose
Water	
Heated to 90–95°C for 2 h then adjust pH to 6.8 with NaOH	

Pork Type Flavor	
L-Cysteine HCl	Wheat gluten hydrolysate
D-xylose	
Heat at reflux temperatures for 60 min	

Bacon Type Flavor	
L-cysteine HCL	Thiamin HCL
Liquid soy hydrolysate	D-xylose
Smoke flavor	Bacon fat
Cook at reflux temperatures for 90 min	

Source: From Manley, C.H., Flavor Interactions Workshop, IFT sponsored, Orlando, FL, 2003. With permission.

9.3.2.3 Final Flavor

The process flavor may be sold as a paste or as a powder. The paste often separates into a bottom layer of insoluble solids, a middle section of solution/suspension, and a top layer of solid fat. This striation makes it difficult to obtain a uniform sample/aliquot from the container. The preferred product form is a powder. Powders are easier to handle and store, have a longer shelf life, and are easier to apply in dry applications. As noted earlier, however, these materials are difficult to dry and prevent from caking during storage.

One can see that this overall process is very much an art and that the exact duplication of a competitive flavor is extremely difficult. It generally is difficult to determine initial reaction system composition due to the consumption of the reactants, plus there is little information that can be gathered about processing solids content, pH, times, or temperatures of heating, all critical factors in determining flavor, from examining the finished flavoring.

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9.3.3 Hydrolyzed Vegetable Proteins (HVP)

The production of high quality hydrolyzed plant proteins and yeast autolysates has served as the basis of most commercial process meat-like flavorings for many years [37]. These products are offered as single components for direct use in food mixes or blended with flavor potentiators (e.g., MSG, 5′-nucleotides) and, often, spices. Currently, there is a wide range of such hydrolyzates produced in the market, each claiming specific color and flavoring characteristics, which in turn are dependent on the source protein (e.g., wheat gluten, maize gluten, soybean flour, etc.). The biggest proportion of HVP is based on soybean protein [38].

The three available industrial methods for the production of HVPs are (i) enzymatic: a slow process that may result in the formation of bitter peptides and typically lacks the desired aroma/taste profile [20,21,39]; (ii) alkaline hydrolysis: which typically results in unacceptable flavor profile and an unbalanced amino acid content, and (iii) acid hydrolysis: the most preferred method that is cost effective and yields a range of good flavors (see Chapter 11, section 11.4.1.2 Hydrolyzed Vegetable Proteins).

Complete hydrolysis of the protein is desirable using a high hydrochloric acid to protein ratio to ensure minimal amino acid degradation and a better flavor profile (as well as minimize any allergy issues). However, a large excess of acid has to be avoided as its neutralization would result in an abnormally high salt content in the finished product. Charcoal filtration of the neutralized aqueous solution results in the partial removal of certain amino acids as well as some unwanted impurities. The degree of filtration has to be carefully controlled as this determines the level of color in the end product.

If the starting material is high in glutamic acid, then the resulting HVP will have marked flavor potentiating properties. Some commercial HVPs are essentially free from reducing sugars so that further heating as part of a food system does not significantly enhance any Maillard reaction that may take place (other than contributing amino acids). Others are high in polysaccharides and give a decided roast meat or even burnt character on further heat processing. These differences underline the need to evaluate HVPs under actual processing conditions so as to determine their precise flavor contribution. The manufacturer's literature and specifications should be carefully studied before using these flavoring ingredients.

9.3.4 Autolyzed Yeast Extracts (AYE)

This is the product of the self-digestion of yeast cells, achieved by heating an aqueous slurry of active yeast at 45°C that effectively kills the cells without damaging any enzymes present. These enzymes are mostly proteases, which attack the cell components, partially solubilizing them. The resulting low molecular weight compounds include peptides, amino acids, and nucleotides. The degree of autolysis depends on the temperature/time conditions and the pH of the medium. The process is terminated by raising the temperature in order to inactivate the enzyme system. The degree of protein and carbohydrate breakdown can be varied to satisfy particular flavor and handling characteristics.

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The flavor of the end product is determined, to a large extent, by the choice of yeast and the nature of the substrate. Aerobically grown *Saccharomyces cerevisiae* gives a much stronger, more beef-like flavor than anaerobically grown *Saccharomyces carlsbergensis*.

Hydrolysis with hydrochloric acid at 100°C is a more effective method of solubilizing yeast than autolysis. The conditions used are similar to those employed for the production of HVP (see Chapter 11, section 11.4.1.2 Hydrolyzed Vegetable Proteins and section 11.4.1.1 Yeast Extracts). When the hydrolysis has reached the desired level, the reaction liquor is neutralized to pH 5.5, clarified to remove all solid matter and partially concentrated. In some grades of yeast extract, the cell wall tissue is retained, the higher glycan content making these effective emulsifiers and thickeners. Further purification involves using either adsorption or ion-exchange techniques to remove undesirable components. The purified liquor is then finally concentrated and may be spray dried. Autolyzed yeast and hydrolyzed yeast extracts are excellent savory flavoring agents as well as contributors to the nutritive value of an end product. The industry has developed a line of such products that have been thermally processed to provide base meaty flavors.

9.4 ENZYMATICALLY DERIVED FLAVORINGS

9.4.1 Introduction

Enzymes find use in several areas of the flavor industry, including processing aids (e.g., free essential oils from plant cell structures, e.g., Sakho et al. [40]), for the liberation of flavor compounds from nonvolatile precursors (e.g., glycosides or cysteine conjugates, e.g., Winterhalter and Skouroumounis [41]), and the conversion of a precursor to a desired flavorant (biotransformations, Berger et al. [42]). In this chapter, enzymes will initially be discussed as they are used to enhance the potency of dairy flavorings. Later in this chapter (in the biotechnology section, 9.7) their use in producing pure aroma chemicals (catalysts for biotransformations) will be discussed.

Dairy products are typically low in flavor and are a high-cost ingredient. Thus, there is a strong incentive for the development of more potent dairy flavors to provide more cost-effective ingredients to the food industry. Increased flavor potency is generally achieved through one, or a combination of, the following processes:

1. Enzymatic modification (EM) of the base dairy product (controlled lipolytic and/or proteolytic enzymic action on fresh milks, creams, butters and butterfats, and cheeses)
2. An initial fermentation of the product (e.g., EM sour cream vs. EM fresh cream) followed by enzyme modification
3. The addition of other natural flavors (e.g., starter distillate, natural butyric acid, or methyl ketones)
4. Controlled high temperature processing (thermally processed to obtain browned flavor) [43]

Enzyme modified dairy products as a group are typically used to enhance or strengthen a dairy flavor as opposed to completely replacing the dairy product. They

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are approximately 15 to 30 times more intense than the corresponding non-EM product, and thus find usage at 0.1 to 2% in finished applications [44]. Unfortunately, EM products lack a complete flavor profile, and thus become harsh or unnatural when used at high levels. In EM processes, manufacturers primarily use lipolytic enzymes, which produce free fatty acids. These acids provide only a part of the characteristic flavor profile of a dairy product and thus become unnatural at high levels. While more complete flavor profiles may be obtained by using additional enzymes (e.g., proteases, peptidases, and esterases), the flavor profile is still lacking.

Some basic properties of enzymatic reactions will be discussed as will some of the existing EM dairy products and modifications thereof. Reviews by Kilcawley et al. [44], Freund [45,46], and Klein and Lortal [47] on EM dairy products are recommended for detail.

9.4.2 Properties of Enzyme Catalyzed Reactions

Enzymes are complex proteins that act as catalysts in various biochemical interactions. They generally function at ambient temperatures, are specific in action, have an optimum pH, and are effective at very low concentrations. A brief discussion of some of the general properties of enzyme catalyzed reactions follows below.

The optimum temperature for the activity of an enzyme as well as lower and upper temperature limits are unique to each enzyme. Some enzymes are extremely heat stable while others are easily denatured. The plot shown in Figure 9.1 illustrates a typical relationship between enzyme activity and reaction rate. Initially, the reaction rate increases with temperature, but at some temperature, the enzyme starts to denature, thereby losing activity. If one is using enzymes to generate a flavor, one chooses to use the optimum reaction temperature to minimize processing time.

Each enzyme also has a unique optimum pH for activity (see example in Figure 9.2). There is an optimum because enzyme structure is pH dependent and outside a

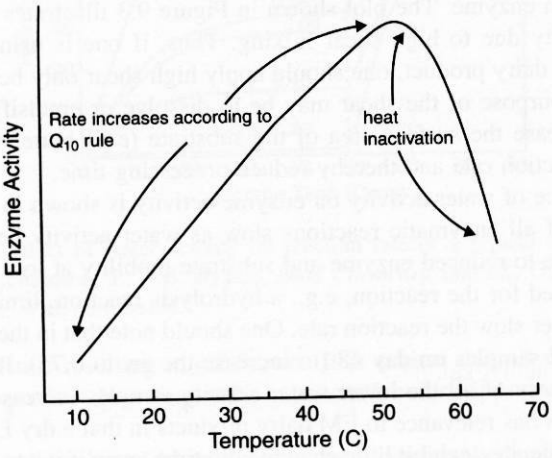

FIGURE 9.1 The influence of temperature on reaction rate of a typical enzyme catalyzed reaction. (From Mathewson, P.R. *Enzymes*, Eagan Press, St. Paul, 2004. With permission.)

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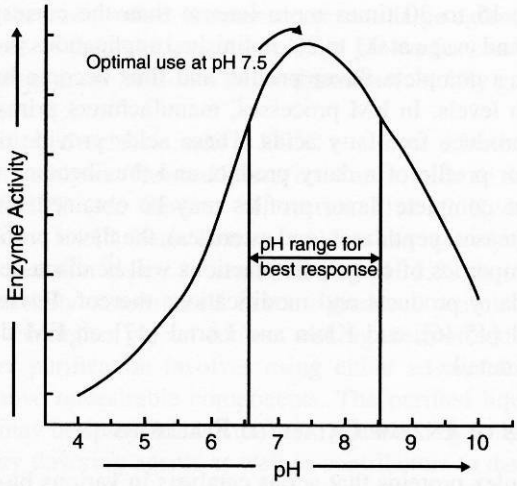

FIGURE 9.2 The effect of pH on reaction rate of a typical enzyme catalyzed reaction. (From Mathewson, P.R. *Enzymes*, Eagan Press, St. Paul, 2004. With permission.)

given pH range, the enzyme begins to denature and thereby loses its activity. One often illustrates the activity of an enzyme in terms of a lock and key drawing. The substrate is the key that must fit (or orient) itself into the enzyme (the lock) in the proper manner or the enzyme cannot act upon it. Denaturation is viewed as a change in structure of the enzyme resulting in a loss in enzyme activity. The pH optimum as well as working pH range is again enzyme dependent. Enzymes derived from acid sources (e.g., bromelin, a protease from pineapple) will tend to have very low pH optima while those isolated from a neutral or alkaline environment (lower intestine, egg white, or fish muscle) would have optimal pHs in the alkaline region.

Many factors can denature, i.e., inactivate, enzymes. Shear can be very effective in denaturing an enzyme. The plot shown in Figure 9.3 illustrates how an enzyme may lose activity due to high shear mixing. Thus, if one is using an enzyme to produce an EM dairy product, one should apply high shear only before the enzyme is added. The purpose of the shear may be to dissolve or emulsify the base dairy product to increase the surface area of the substrate (e.g., butter fat). This would enhance the reaction rate and thereby reduce processing time.

The influence of water activity on enzyme activity is shown in Figure 9.4. The reaction rates of all enzymatic reactions slow as water activity becomes limiting. This may be due to reduced enzyme and substrate mobility at low water activities. If water is needed for the reaction, e.g., a hydrolysis reaction, limited water availability will further slow the reaction rate. One should note that in the example, water was added to all samples on day 48 (to increase the aw to 0.75). It is relevant that the enzyme activity of all the lower water activity samples increased back to their full activity. This has relevance to EM dairy products in that a dry EM product may appear to be stable, i.e., exhibit little change with time, even if it has active enzymes. However, if the active dry product is given the opportunity to gain moisture, e.g., added to a high moisture food, the enzymes may continue to attack the dairy product

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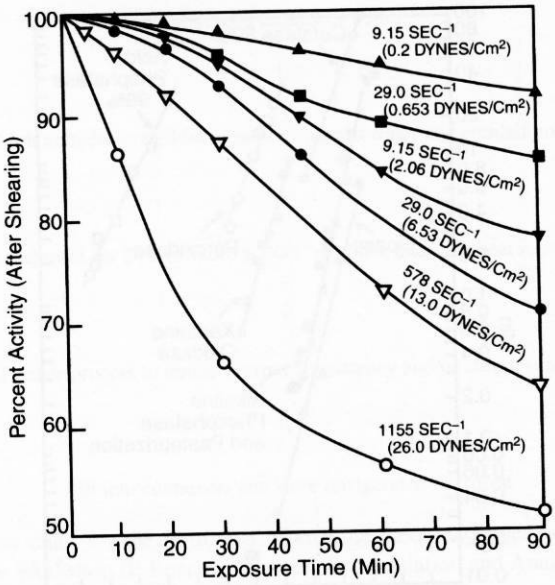

FIGURE 9.3 The effect of shear on the inactivation of a typical enzyme. (From Richardson, T., D.B. Hyslop, *Food Chemistry,* 2nd ed., O.R. Fennema, Ed., Dekker, New York. With permission.)

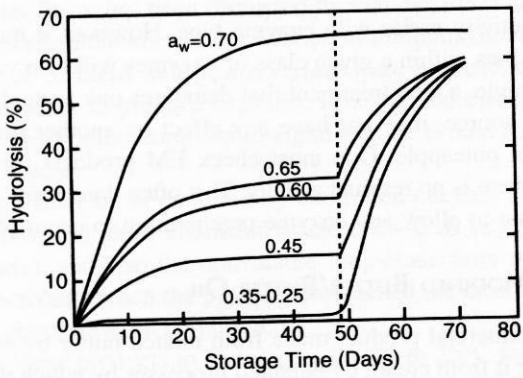

FIGURE 9.4 The effect of water activity on reaction rate of a typical enzyme catalyzed reaction. (From Richardson, T., D.B. Hyslop, *Food Chemistry,* 2nd ed., O.R. Fennema, Ed., Dekker, New York. With permission.)

base or the foodstuff resulting in off-flavors. It is critical that all enzymes used in making an EM product be completely inactivated before it is used in a finished application.

Enzymes used in making EM products are most commonly denatured by heating. It is critical to know the heat tolerance of the enzymes used in a product so that an adequate heat treatment may be applied. The plots provided in Figure 9.5 show how

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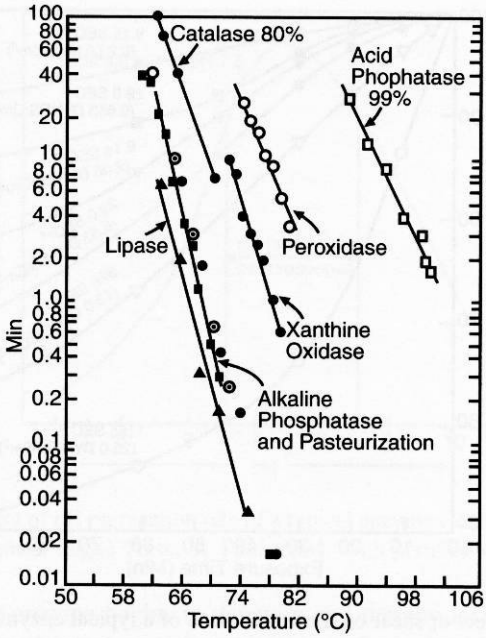

FIGURE 9.5 The effect of temperature/time on the inactivation of a typical enzyme. (From Richardson, T., D.B. Hyslop, *Food Chemistry,* 2nd ed., O.R. Fennema, Ed., Dekker, New York. With permission.)

greatly enzyme activity varies with enzyme type. However, it must be further recognized that enzymes within a given class of enzymes will also vary greatly in heat stability. For example, a heat treatment that denatures one proteolytic enzyme, e.g., from a microbial source, may not have any effect on another proteolytic enzyme, e.g., one found in pineapple. One must check EM products for residual enzyme activity to insure there is no residual activity. This often done some period (e.g., 72 h) after manufacturing to allow any enzyme regeneration to occur [48].

9.4.3 Enzyme Modified Butter/Butter Oil

Butter oil is a commercial product made from melted butter by either centrifuging or directly making it from cream by patented processes by which the unwanted curd is removed by running the nearly churned cream into hot water. This causes the curd to precipitate, and the butter oil can then be recovered by centrifuging. Butter oil can be used directly in many food products or may be treated with selected enzymes (or organisms), which bring about lypolysis. The resulting very flavorful product is known as EM butter oil (or lipolyzed butter oil, LBO).

The commercial production of EM butter oil has been described by Andres [49] and the making of a dry-powdered version by low-temperature, spray-mat, drying is discussed by Farnham et al. [50]. These products offer richer, more intensified butter flavor profiles that have wide applications, particularly in baked goods and

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Anhydrous milkfat or butter

↓

Melt anhydrous milkfat or butter, and add water and emulsifiers

↓

Add lipase enzyme and react for 24 h or more at 30°C or above and then heat inactivate added enzyme

↓

Further process to obtain desired consistency and/or composition

↓

Fill into containers and store refrigerated or frozen

FIGURE 9.6 Flow chart for the formation of enzyme modified butter or butter oil. (From Freund, P., Flavor Workshop II: Reaction Flavors, Encapsulation and Analysis, University of Minnesota, St. Paul, September 2003. With permission.)

spreads. Very little information has been published on the precise organisms or enzymes employed, but the use of the microorganisms *Trametes sanguinea* has been patented [51]. The efficacy of *Streptococcus lactis* subsp. *diacetylactis* in the production of the butter flavor has been discussed by Piatkiewicz et al. [52], and Olson et al. [53] have described the microencapsulation of multienzyme systems. Aqueous cell free extracts of S. *lactis* subsp. *diacetylactis* are emulsified in milk fat and extruded into cold water to produce capsules. It is claimed that these products are effective in significantly increasing, up to eightfold, the production of diacetyl and acetoin in young cheese.

Commercially, butter oil is modified by controlled enzyme action that releases the volatile and flavorful short chain fatty acids, C4–C10 (e.g., butyric, caproic, caprylic, and capric), and also the nonvolatile long-chain fatty acids. The enzyme system is heat inactivated when the process has reached the desired end point. Such products contain about 48% fat (Figure 9.6).

It is usual for these products to be tempered at 20 to 25°C for 12 h prior to use to make them easier to incorporate into food mixes and to allow flavor equilibrium to be achieved. End products containing them should not be submitted to sensory assessment for a period of 24 to 48 h after manufacture. Manufacturers recommend that LBOs should be stored at 2 to 6°C and be used within 6 months from date of manufacture.

9.4.4 Enzyme-Modified Cheese (EMC)

Cheese flavor is very important in many savory food and snack products. In some instances an adequate flavor level can be achieved by the careful selection and use

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of natural cheeses, using the powder form if necessary, but in many instances the percentage needed to attain the correct flavor is not technologically acceptable and frequently is uneconomical. In such products, more intensely flavored cheese flavorings are required, and the industry has produced these by the application of enzyme technology.

EM Cheddar, Swiss, Romano, Mozzarella, Provolone, Feta, Parmesan, Blue, Gouda, Gruyere, Colby, and Brick cheeses are commercially available [44]. Kilcawley et al. [44] have provided a thorough review of this topic and it is highly recommended for further reading.

9.4.4.1 Enzymes Used

The key to producing Enzyme-modified cheeses (EMCs) is the proper choice of enzymes and their application under optimal conditions. The majority of research in this area has been on the lipase enzymes. A wide variety of lipase enzymes are commercially available. These enzymes come primarily from animal or microbial sources. Lipase choice is critical because each lipase has a specificity for hydrolysis thereby determining the free fatty acid profile (a key determinant of flavor) in the EMC [54].

Animal lipases are derived primarily from bovine or porcine pancreatic sources, and the pregastric tissues of kid goat, lamb, and calf [55]. The pregastric (i.e., oral) lipases (PGE) have a high specificity for the short chain fatty acids that characterize most of the natural cheeses and thus are preferred enzymes. Most PGEs have an optimum pH ranging from 4.8 to 5.5 and optimum temperature ranging from 32 to 40°C. Birschbach [56] has described the flavor character generated by different PGEs as follows:

Calf PGE – buttery and slightly peppery flavor
Kid PGE – sharp peppery often called piccante flavor, which quickly clears from the palate
Lamb PGE – peccorino flavor described as "dirty sock"

The use of any animal source enzyme brings concern for religious (Kosher or Halal) or dietary choices (e.g., vegetarian). These concerns as well as cost issues have promoted the development of microbial lipases and esterases for this purpose. If these enzymes are approved as GRAS (Generally Recognized as Safe), they can be used in this application.

As one would expect, microbial enzymes range in specificity. For example, lipases from *Candida cylindracea* and *Staph aureus* have little specificity while those from *Geotrichum candidum* are specific for long chain acids [57], which are of little value in this application. Fortunately, most microbial lipases show specificity for the one and three positions of the triglyceride where the short chain fatty acids are found. While substantial information exists in the literature characterizing lipase enzyme activity to help in enzyme choice [58], enzymes must be evaluated for function in a model system. This often is done in a curd slurry system, which is produced by blending two parts of fresh cheese curd plus one part sterile dilute salt solution (5.2%

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NaCl), aspirating with nitrogen (5 min) and then inoculation with enzyme [59]. The system is then incubated at 30°C for 4 to 5 days, with the flavor periodically evaluated.

While lipases play a major role in determining the flavor of many cheeses, proteolytic activities are often considered more important to the flavor of some hard cheeses, e.g., Cheddar. The use of proteinases becomes problematic since these enzymes often produce bitter peptides. Thus, the manufacturer typically has to use a balance of proteinases and peptidases: the peptidases are necessary to break down the bitter peptides [59].

9.4.4.2 General Processes Employed

EMCs are generally manufactured from cheese pastes that are made from the cheese of the same type. Additional components such as butterfat or cream may be added to add extra precursors when appropriate. Noncheese ingredients such as MSG, yeast extract, diacetyl, or other flavorants may also be added, but they may have to be declared on the label of the final product. Consistency in this base material is critical to the production of a standardized EMC product. Off-flavors may develop during incubation of the paste/enzyme slurry since the conditions are optimal for microbial growth. Equipment must be sterilized and precautions taken to prohibit microbial contamination. Bacterial inhibitors such as nitrates, sorbate, or nicin may be used. Free fatty acids generated by lipase enzymes afford some inhibition. Incubation time and temperature influence enzyme action and must be carefully controlled.

As noted earlier, the inactivation of residual enzymes to terminate the reaction is critical to flavor stability. The heat treatment must be adequate to inactivate the enzymes but not cause undesirable flavor changes. One should note that commercial enzyme preparations used in manufacturing may contain enzymes other than the primary enzyme(s): these are crude preparations as opposed to reagent grade enzyme preparations. For example, West [48] noted that pancreatic lipase preparations may contain amylases that can cause undesirable changes in flavor and texture in the finished product if not inactivated.

Kilcawley et al. [44] have outlined several industrial procedures for the production of EMCs. One example is presented in Figure 9.7.

It is claimed that the use of EMCs can result in considerable raw material cost savings since up to 50% of cheese solids in many product formulations can be replaced by a small quantity of EMC. Their use also enables food processors to adjust any variable flavor in natural cheese included in their product. They are of particular value in (a) processed cheese, (b) cheese powders, (c) cheese snacks, (d) pizza sauce, (e) cheese biscuits, (f) cheese spreads, (g) quiche, and (h) dips. The rate of addition is directly related to the profile of the natural cheese present and the strength required in the end product. On average, the recommended use rate is 1 to 3% of the total recipe weight. EMCs should be stored at 2 to 6°C and used within 6 months from date of manufacture.

Similar to the process flavors discussed earlier, EM dairy products are the result of the application of science plus substantial trial and error (i.e., art). Flavor character is dependent the starting materials, the enzymes added, the incubation conditions,

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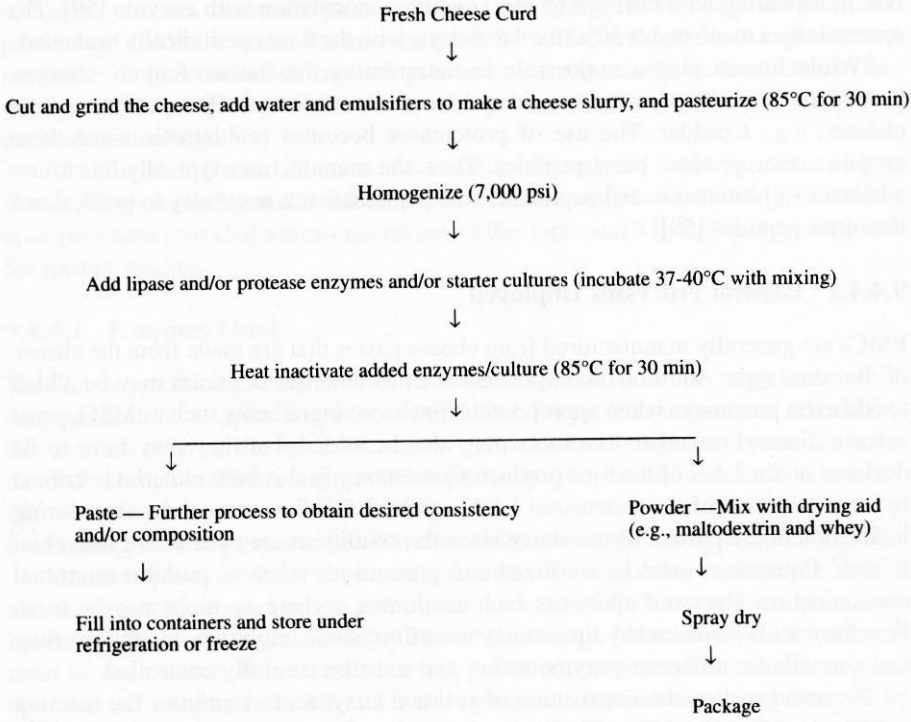

FIGURE 9.7 Flow chart for the formation of enzyme modified cheese. (From Kilcawley, K.N., M.G. Wilkinson, P.F. Fox, *Int. Dairy J.*, 8, 1998. With permission.)

and virtually all steps of processing. This makes them extremely difficult for competitors to duplicate.

9.4.5 Further Processed EM Dairy Products

The flavor of an EM dairy product can be further manipulated to provide other sensory profiles. A simple way to do this is through an initial fermentation, i.e., prior to EM. A fermentation can strengthen the flavoring plus add other notes not added by the enzyme treatment. Thus a more complete flavor profile can be achieved. The cultures used in fermentation are typically the same as those used in a cultured dairy product.

Many of these products can be further enhanced by the use of top noting. This is similar to the process as it is applied to meat-like flavorings. Pure chemicals are added to the EM dairy product to provide notes not obtained in subsequent EM treatment. This process can greatly strengthen and round out an EM dairy flavoring. The availability of numerous natural dairy flavor chemicals often permits these top noted products to carry a natural label.

The final process applied to EM dairy products is thermal processing. Essentially, one applies a process similar to that of making a process flavoring (meat, chocolate,

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or coffee) to dairy products. The EM dairy product may have added ingredients prior to thermal processing to promote certain reactions, e.g., the Maillard reaction. Since dairy products are widely used as ingredients for the baking industry, it is logical that heated EM dairy products are very useful ingredients for the baking industry.

9.5 FLAVORS MADE BY FERMENTATION

The word *fermentation* (from the Latin *fermentare* meaning to cause to rise) was first applied to the making of wines and beer, processes whose origins are lost in history. It is now used to describe enzyme-catalyzed reactions in which compounds such as carbohydrates are broken down anaerobically by bacterial or yeast enzymes, leading to the formation of pyruvic acid (and other end products). In the case of yeast, this intermediate is split into carbon dioxide and acetaldehyde, which in turn is converted into ethanol. Industrially, fermentation is carried out using carefully selected microorganisms appropriate to the substrate, under precisely specified conditions. The products are wide ranging and include alcohol, glycerol, butyl alcohol, acetone, as well as lactic, acetic, citric, gluconic, and glutamic acids, many of which are important in food flavorings.

9.5.1 Yeasts

Yeast is a microscopic, unicellular organism of which there are several hundred species, only a very few of which are of commercial importance, namely, *Saccharomyces cerevisiae, Saccharomyces carlsbergensis, Candida utilis* (formerly *Torula utilis), Candida tropicalis,* and *Kluyveromyces fragilis.* Selected *Saccharomyces* spp. are used for the production of wines and other alcoholic beverages.

The choice of yeast depends on the nature of the substrate in which it is to be grown. S. *cerevisiae* and S. *carlsbergensis* utilize glucose, galactose, maltose, and sucrose; *Candida utilis* (the so-called torula yeast) prefers pentose sugars such as arabinose and xylose and is widely used in the fermentation of molasses and sulfite waste liquors; *Kluyveromyces fragilis* grows on lactose contained in dairy byproducts.

9.5.2 Vinegar/Acetic Acid

Vinegar has a long and useful history both as a condiment and as a food preservative. It is the product of the alcoholic and acetic fermentation of a sugar-containing solution without any intermediate distillation except in the production of spirit vinegar. Numerous vinegars are available for use in food processing. These include (i) wine vinegar, the alcoholic fermentation being from grapes; (ii) malt vinegar, made from malted barley; (iii) spirit vinegar, prepared by the acetic fermentation of distilled alcohol, itself made by fermentation; (iv) fruit wine vinegars, made from grape and nongrape fruit wines; (v) cider vinegar, made from fermented apple juice; and (vi) distilled vinegar, obtained by the distillation of malt vinegar. In recent years, the variety of vinegars has grown immensely [60]. The flavor characters of these vinegars vary considerably.

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Two entirely different fermentations are involved in the manufacture of vinegar:

1. Fermentation of sugar by yeast:

$$\text{hexose} \rightarrow \text{ethanol} + 2\ CO_2$$

2. Conversion of alcohol to acetic acid by the action of acetobacter organisms:

$$\text{ethanol} + 2O_2 \rightarrow \text{acetic acid} + 2\ \text{water}$$

The process is usually carried out within the temperature range 24 to 27°C. The alcoholic fermentation is induced by a strain of *Saccharomyces cerevisiae* var. *ellipsoides,* which has been grown in the presence of a minimum level of sulfur dioxide to eliminate extraneous organisms.

Using a substrate of 8 to 20% fermentable sugars, the primary fermentation is carried out in open vats fitted with a cooling system to regulate the temperature. The added yeast, during its normal reproduction, converts the sugars to alcohol and carbon dioxide and, in the case of malt vinegar, similarly converts dextrins obtained from the malted barley. Fermentation takes about 7 days to completion at which stage the product contains 6 to 7% alcohol, which represents about 90% of theoretical conversion of the sugars. After sedimentation of the yeast cells, the supernatant alcoholic liquor is filtered and is ready for the second acetic oxidative fermentation.

This secondary fermentation requires considerable oxygen and is carried out in one of two ways:

1. Packed generator systems. The traditional acetifier comprises a tall vat packed with an inert material (usually birch twigs or wood shavings) which act as the carrier for the acetobacter culture. The alcoholic substrate is sprayed on the top of the column and allowed to flow down against a stream of air, introduced from the bottom of the vat, into a false bottom and is recirculated for several days until most of the alcohol has been oxidized to acetic acid.
2. Fring's acetator. This is a submerged culture fermentation, involving vigorous stirring of the substrate to induce adequate aeration. Several purpose-designed acetators are available, but in each the acetobacter organisms are introduced into the substrate and grow in a suspension of fine air bubbles produced by the considerable upwards turbulence. The process is continued until the desired level of acetic acid is achieved. This type of plant requires less space than the traditional vats and gives a more rapid conversion but is more energy consuming. The resulting vinegar is filtered and pumped into storage vats where it is allowed to mature for several months during which time it develops a delicate and characteristic bouquet.

Federal standards require vinegar to contain not less than 4 g of acetic acid per 100 ml.

Distilled vinegar is prepared by distilling the acetic acid and volatile constituents from a normally fermented vinegar. It is colorless and retains much of the characteristic aroma and flavor of the original vinegar. It is of particular use in products where color is an important attribute (e.g., pickles, mayonnaise, tomato ketchup).

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Concentrated vinegar, containing up to about 40% acetic acid, may be prepared by freeze concentration using a scraped-surface Votator heat exchange unit operating at –8°C. The water is removed as ice and most of the aromatics remain in the concentrated product.

Some of the newer vinegars such as balsamic vinegar are made by combining processes. For example, balsamic vinegar is made by cooking down the must (juices) of unfermented grapes (red Lambrusco [used extensively on the Emilian plains], white Spergola and Occhio di Gatta, and/or Berzemino grapes), adding a culture to the reduced must (or back inoculating), allowing fermentation and then aging in selected wood barrels [61,62]. The characteristic flavor of this product is due to a combination of thermal processing (cooking of the must), fermentation, and wood aging — somewhat analogous to the heating of EM dairy products to create new flavorings.

9.5.3 Dried Inactive Yeast Powder

An inevitable and commercially important byproduct of fermentation processes is yeast. This can be recovered, dried, and inactivated to form a valuable food ingredient. Although the brewing industry is the major source, the demand is sufficient to warrant the growing of selected yeasts primarily for this purpose. Henry [63] has described the production of dried Torula yeast, resulting from the fermentation of the waste sulfite liquors from paper production.

The yeast slurry, containing about 1% solids, is continuously separated from the substrate by centrifuging, the collected yeast cells are washed to remove all adhering chemicals and then dried to a moisture content of <7% to give the commercial product. This product has a very high protein content (min. 45%) and substantial amounts of B vitamins. The USF XIII confines the source to either *S. cerevisiae* or *Candida utilis*.

Dried inactive yeasts are used not only as valuable nutrient and vitamin supplements but in the flavoring of dry savory seasonings, dip mixes, gravy and sauce mixes, snack products, etc., to which they give a rich, full-bodied flavor character. Because of the many variables that affect the flavor profile of inactivated yeast, the products of different manufacturers have quite distinctive flavoring properties. The profile may be characterized by such nuances as sweet, caramel-like, roasted, beefy, cheesey, or creamy, and this implies that such products must be individually assessed prior to use.

9.6 FLAVORS MADE BY PYROLYSIS: SMOKE FLAVORS

9.6.1 The Smoking of Foods

The process of curing and smoking food, which has evolved slowly over thousands of years, was probably discovered by accident when it was observed that meats and fish hung up to dry in a smoky atmosphere remained edible for longer periods and also acquired a pleasant taste. For many hundreds of years the process has been carried out as a means of preserving both meat and fish, although the emphasis has gradually changed from preservation to the imparting of a very pleasant flavor to the food product.

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Initially, the smoking process was carried out in a kiln with little or no control over the smoking process. Today, smoke generators are designed to ensure the burning of hardwood sawdust under controlled conditions of forced air recycling and temperature. Even so, the slow burning of wood demands considerable skill and may give rise to products of variable flavor quality. The direct use of wood smoke is relatively inefficient, results in messy tarry deposits throughout the smoke generating plant, and often results in the deposition of unsightly particulate matter on the surface of the smoked product. In spite of these problems the smoking of meats and fish is still extensively used, but it is for these reasons that the use of natural liquid smoke flavors have gained wide acceptance.

9.6.2 Natural Liquid Smoke Flavorings

The Code of Practice issued by the IOFI [64] specifies smoke flavors as follows:

"Smoke flavors are concentrated preparations, not obtained from smoked food materials, used for the purpose of imparting smoke type flavor to foods. They are prepared according to one or more of the following methods:

1. By subjecting various untreated hardwoods to processes described as (a) controlled burning, or (b) dry distillation at appropriate temperatures, usually between 300 and 800°C, or (c) treatment with superheated steam at temperatures usually between 300 and 500°C, and condensation and capturing of those fractions which have the desired flavor potential
2. By applying further isolation techniques to the fractions obtained under 1. [item] 1 in order to retain only the flavorwise important fractions or components
3. By compounding chemically defined flavoring substances. Smoke flavors should contribute no more than 0.03 ppb 3,4-benzopyrene to the final food product."

9.6.3 Pyroligneous Acid

When wood is heated without an excess of air to temperatures in excess of 250°C, it decomposes into charcoal and a volatile fraction, which condenses on cooling. The aqueous phase of this condensate is known as pyroligneous acid. The chief component is water containing about 10% of acids, mainly formic, acetic and propionic.

9.6.4 Smoke Condensates

These are prepared by drawing the distillation and combustion products from damp hardwood sawdust into a condenser fitted with an electrostatic precipitator. This removes all particulate matter from the condensate, which is collected and settled to allow separation into aqueous and tarry phases. The clear aqueous phase is the desired product.

Modern generators produce smoke under carefully controlled conditions of combustion temperature and air flow. Sawdusts of hickory, maple, and oak woods

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are used as the source material. The smoke passes through a settling chamber to remove heavy tars and wood ash and is then directed upwards through a column of inert ceramic material countercurrent to a steady flow of water. This is recycled as necessary until the titratable acidity is 5 to 12%, as acetic acid. The product is then filtered and set aside to age, during which time it loses its unpleasantly sharp flavor character and additional tarry matter deposits.

More recent manufacturing processes consist of inclined rotary calciners. Sawdust is fed continuously into one end of the inclined tube and discharged at the other end as charcoal. The vapors are handled as already described. The rate of feed, inclination of the tube, and rate of air flow are optimized to give a product with the best smoke flavor and the lowest content of polycyclic aromatics.

9.7 BIOTECHNOLOGY TO PRODUCE FLAVORING MATERIALS

9.7.1 Introduction

The flavor market in the U.S. (and world) has been heavily weighted toward natural flavorings for the last 30+ years. Artificial flavorings find use today in lower cost food products or in situations where natural flavoring counterparts do not exist (e.g., top noting of meat-like flavorings). The market demand for natural flavorings has resulted in a great deal of research directed at developing processes for making natural flavoring chemicals. Gatfield [64] estimated that approximately 100 natural flavor components are being produced through biotechnology.

The classification of a flavoring (or flavor chemical) as being natural has been defined by the FDA and published in the CFR (presented earlier). It is adequate here to note that any product of fermentation or enzymatic activity starting with a natural precursor(s) yields a natural product according to this definition. Gatfield [65] commented that it would also seem reasonable that only those compounds found in nature could be considered natural for it may be possible to make compounds not found in nature through these approved techniques. In this chapter we will focus on examples of using enzymology and fermentation to yield natural flavoring chemicals or flavorings. Texts including those edited by Parliment and Croteau [66] and authored by Berger [67] as well as a textbook chapter by Berger et al. [42] are all highly recommended. Several comprehensive reviews are also available [68–70].

9.7.2 Production of Natural Flavoring Materials by Enzymatic Action

There is little question that enzymes exist in nature to accomplish nearly any chemical conversion that one would desire. Thus, theoretically, one could find an enzyme (or series of enzymes) to convert some precursor to any related natural flavor compound. However in practice, enzymes have found only a few commercial applications for this type of task (despite an abundance of publications in the literature). Enzyme catalyzed reactions suffer from several practical limitations, including:

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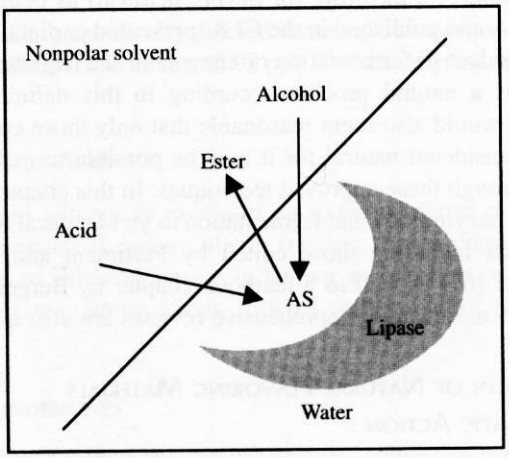
1. Instability and often very short life times in application
2. Often require cofactors, e.g., NAD or ATP
3. Are slow and offer a lower yield than a chemical reaction
4. Are expensive, especially when batch processes are employed
5. Catalyze only a single step of a reaction

9.7.2.1 Ester Formation

The most widely used enzymatic bioconversion used in the flavor industry is the condensation of natural acids (generally acetic or other short chain acids) and alcohols (generally ethanol or fusel alcohols) to yield a wide range of esters. It is recognized that enzymes typically are reversible and thus, lipases (or esterases) normally associated with the *hydrolysis* of esters, can be used to *synthesize* esters under certain conditions.

Numerous researchers have published on the formation of esters using enzymes in nonpolar solvents (see Gatfield [71,72]). The diagram in Figure 9.8 illustrates how conducting enzymatic reactions in an organic solvent can reverse its action [73]. In this case, the enzyme is hydrated and thus presents an aqueous environment that accumulates high concentrations of free acids and alcohols due to their solubility in aqueous systems. The high concentration of the acids and alcohols favors their condensation into esters. The esters are less polar and thus go into the organic phase depleting the ester in the enzyme environment. This drives the equilibrium toward the further formation of esters. As one might anticipate based on this partitioning, the use of nonpolar solvents often will also increase reaction rate and yield.



Ester + water ⇌ alcohol + acid

FIGURE 9.8 Enzymatically catalyzed synthesis of esters. (From Armstrong, D.W., B. Gillies, H. Yamazaki, *Flavor Chemistry: Trends and Developments*, R. Teranishi, R.G. Buttery, F. Shahidi, Eds., Amer. Chem. Soc, Washington, D.C., 1989. With permission.)

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A very similar process is used to catalyze the oxidation of aromatic and aliphatic alcohols to their corresponding aldehydes. An alcohol oxidase enzyme isolated from *Pichia pastoris* placed in a two-phase system will readily produce benzaldehyde from benzyl alcohol. While this oxidation is slow and inefficient in aqueous systems, oxidation in a two-phase system increased yield by a factor of nine and reaction time was shortened greatly [74].

It is interesting that industrial byproducts such as fusel oils (distillation byproducts of alcoholic beverages when making high proof products, Table 9.4) were once a disposal problem but now have become valuable starting components for enzymatic conversions. There are few good sources of branched chain alcohols other than the fusel alcohols. The bioconversions of selected amino acids will yield branched chain alcohols and acids, but this is a tedious process in itself. The bioconversion of a fusel alcohol to an acid, and ultimately its esterification to form cis-3-hexenyl-2-methyl butyrate, is shown in Figure 9.9. In this case, the oxidation of 2-methyl-1-butanol is likely catalyzed by immobilized cells of certain Gluconobacter strains as opposed to being enzyme catalyzed. Gatfield et al. [75] have shown that this organism is an effective oxidant of fusel alcohols. Gatfield [65] has also noted that some yeasts can also catalyze such oxidations [76].

Welsch et al. [69] have provided a very comprehensive review of the enzymatic synthesis of flavor molecules. They have provided extensive referencing as well as

TABLE 9.4
Typical Fusel Oil Composition

Alcohol	% Composition
Ethyl alcohol	5
Propyl alcohol	12
Isobutyl alcohol	15
Amyl alcohol isomers	63
Residue	5

2-methyl-1-butanol (fusel alcohol component)
↓
Oxidized biologically
↓
2-methyl butyric acid
↓
Enzymatically esterified
↓
Cis-3-hexenyl-2-methyl butyrate
(key fruit compound)

FIGURE 9.9 Enzymatic conversion of a fusel alcohol to a fruity ester. (From Candella, C., Personal communication, Robertet Flavors, S.A., Grasse, F.R., 1995. With permission.)

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detailed discussions of some fundamental considerations in making these bioconversions. This reference is highly recommended.

9.7.2.2 Resolution of Racemic Mixtures

Enzymatic conversions have also found application in the resolution of racemic mixtures. When an organic synthesis involves a chiral center, one typically obtains a racemic mixture of the end products. Very commonly, chiral isomers differ in sensory properties, and one desires one enantiomer as opposed to the racemic mixture, e.g., L-menthol is the desired form of menthol. It is very difficult to separate enantiomers by chemical means on a commercial basis (cyclodextrin-based columns have some utility in this application). However, a characteristic of enzymatic reactions is their stereospecificity, i.e., they will act on one enantiomer but not another. Thus, enzymatic processes may be incorporated into chemical synthesis to obtain a pure optical isomer. This may be done in either of two ways.

In one approach, an ester of one chiral isomer (e.g., L-menthol) may preferentially be formed via enzymatic action [78]. Then one only has to separate the initial alcohol form (e.g., D-menthol — not esterified) of the target compound from its ester form (L-menthol ester), which is reasonably easy. The alternative approach is to chemically form esters of both enantiomers and use an enzyme to cleave the ester from one chiral form. This again leaves a mixture that is an ester and an alcohol that are readily separated. Berger et al. [42] have illustrated this process for the separation of enantiomers of karahanaenol (Figure 9.10). Gatfield et al. [79] have a recent patent on this method for the isolation of pure L-menthol.

9.7.3 Production of Natural Flavoring Materials by Microbial Action

Unlike enzymatic reactions, microorganisms have the ability to perform multiple reactions, and they do not require cofacors for regeneration (albeit they require nutrients). They may be used to generate a flavor compound from a nonvolatile precursor (e.g., produce a lactone from castor oil), to effect the bioconversion of one volatile to another (e.g., valencene to nootkatone), or effect a chiral resolution (a racemic mixture of menthol). The primary limitation of using microoganisms for

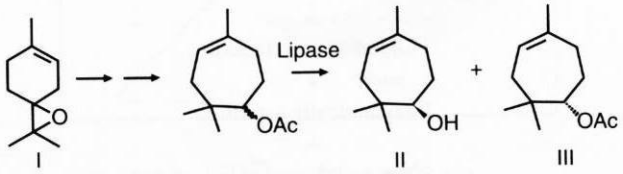

FIGURE 9.10 Enantiospecific alcoholysis of karahanaenol acetate by *Pseudomonas cepacia* lipase. (I) terpinolene oxide; (II) (R)-karahanaenol; and (III) (S)-karahanaenol acetate. (From Roy, A. *J. Agric. Food Chem.*, 47, 12, 1999. With permission.)

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these purposes is that either the precursor or end product may be toxic to the microorganism, and therefore the yields (g/L fermentation broth) may be low and cost of recovery may be high. Thus the use of fermentation to produce natural flavoring materials is largely an economic issue.

9.7.3.1 Fermentation to Produce Flavoring Materials

If one surveys the literature, it is clear that nearly any fruity type flavor could theoretically be produced by fermentation. However, as noted, few of these organisms produce aroma compounds at sufficient concentrations to be economically viable. Researchers try to overcome this problem through both genetic manipulation of the organism and media choice. Genetic manipulation is often done by classic methods such as UV irradiation or chemical mutation (n-nitrosoguanidine). By either method, random mutations occur and a selection is made of those mutants that produce higher levels of the desired end product. Because the mutations are random and hundreds of different mutants may be produced, one of the problems is the isolation and screening of the mutants for the increased production of the target molecule. To illustrate the problem and a possible solution, McIver [80] treated a strain of *Pseudomonas perolens* that produced low levels of 2-methoxy-3-alkyl pyrazines (bell pepper aroma) with n-nitrosoguanidine and selected high yielding mutants for further study. He first plated the organisms, selected pure colonies and grew them in nutrient broth. His screening process was simply smelling the nutrient broth. Those cultures that had an intense odor were analyzed by gas chromatography to determine the actual concentrations of the methoxy alkyl pyrazines. While smelling the tubes is not very scientific, it would have been impossible to analyze each of the cultures by any other method. The cultures possessing the most intense aromas were then grown on different media to enhance volatile production.

As can be seen in Table 9.5, nutrient source had a strong influence on the production of the methoxy pyrazines by a selected mutant of *Pseudomonas perolens*. The end result is that an organism that initially produced a few ppb of the methoxy alkyl pyrazines was converted into an organism that produced ppm levels of the target compounds. While one may not think that ppm levels of an aroma compound is significant, these pyrazines have sensory thresholds in the pptr levels.

Similar approaches (UV irradiation) have been used to enhance the yield of diacetyl in the production of starter distillates [82]. This method yielded cultures that produced a 4.5-fold increase in total acetoin + diacetyl production. Starter distillates are very broadly used in the reproduction of dairy flavors.

One of the earliest and most commercially successful examples of producing flavoring materials by fermentation is the production of 4-decalactone from castor oil (Figure 9.11, [83]). Castor oil is unique in that it is made up of nearly 80% ricinoleic acid (12-hydroxy-9-octadecenoic acid). *Yarrowia lipolytica* initially hydrolyses the ricinoleic acid from the triglyceride and then through β-oxidation, converts this acid to 4-hydroxydecanoic acid. This acid forms a lactone at low pHs to yield the γ-decalactone. The yield on this process is generally considered to be ca. 6 g/L which is very attractive.

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TABLE 9.5
The Production of Selected Methoxy Alkyl Pyrazines by Selected *P. perolens* Mutant on Different Growth Media

Carbon Source	Conc. (% w/v)	2-Methoxy-3-isopropyl pyrazine	2-Methoxy-3-secbutyl pyrazine
Glucose	0.5	2,030[a]	21[a]
	1.0	960	11
	2.0	840	9
Lactate	0.5	6,280	68
	1.0	9,890	112
	2.0	4,730	48
Pyruvate	1.0	11,120	100
Glycerol	No effect at	0.5 or 1.0	
Nutrient broth	No effect		

[a] ppb of compound in growth medium.

Source: From McIver, R.C., Ph.D. thesis, University of Minnesota, St. Paul, 1986. With permission.

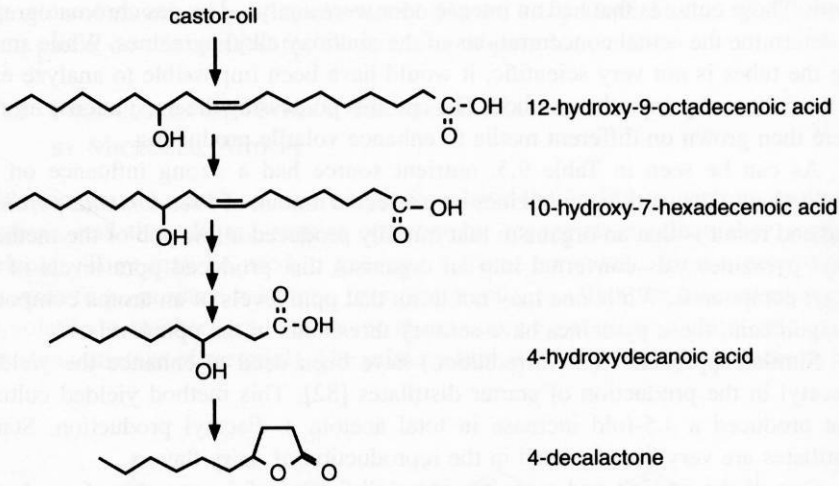

FIGURE 9.11 The conversion of ricinoleic acid to γ-decalactone by *Yarrowia lipolytica*. (From Farbood, M., B.J. Willis, Production of γ-Decalactone, U.S. Patent 4560656, 1985. With permission.)

It is interesting that the δ-decalactone is formed by an entirely different biotransformation. This lactone is formed by the microbial reduction of an α,β-unsaturated lactone found in Massoia bark oil. Massoia bark oil contains about 80% 2-decen-5-olide, 7% 2-dodecen-5-olide and 6% benzyl benzoate. Several microorganisms

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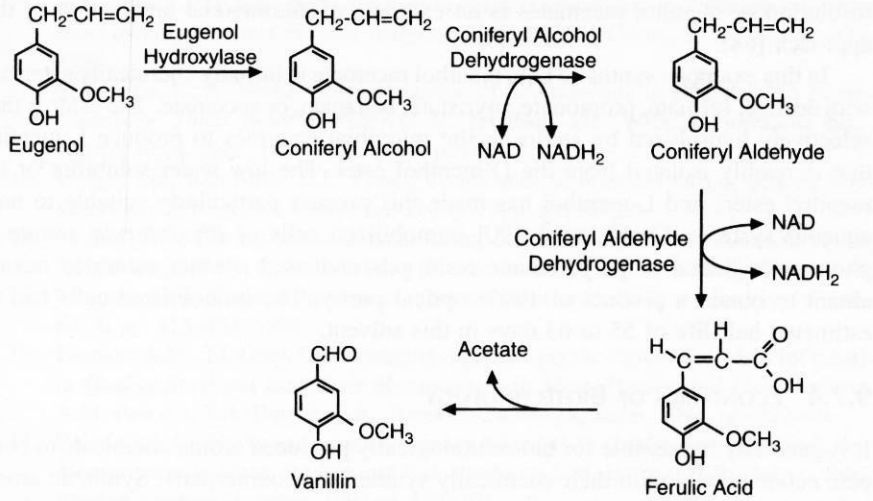

FIGURE 9.12 The bioconversion of ferulic acid to vanillin. (From Gatfield, I.L., *Flavor Chemistry: Thirty Years of Progress*, R. Teranishi, E.L. Wick, I. Hornstein, Eds., Kluwer Acad. Publ., New York, 1999. With permission.)

have been found that will hydrogenate the ring double bond to form the corresponding saturated δ-lactones [84].

Natural butyric acid is produced by fermentation by growing *Clostridium butyricum* or *C. acetobutyricum* on glucose under anaerobic conditions [85,86]. Through the right choices in pH and growth media, concentrations may range from 1 to 2% in the fermentation broth.

9.7.3.2 Bioconversions Via Microorganisms

There are numerous references to the use of microorganisms for bioconversions [42,67,69]. While many such conversions produce too low yields to be of commercial viability, some have found commercial application. Bioconversions can be very efficient since the microbes generally only have to accomplish a limited number of steps in conversion as opposed to multiple steps in a *de novo* synthesis.

An example of this approach was outlined by Gatfield [65] on the conversion of ferulic acid to vanillin (Figure 9.12). Ferulic is an abundant, inexpensive, natural vanillin precursor recovered from waste streams or from the fermentation of eugenol [87]. While many microbial systems have been found that accomplish the desired bioconversion, Gatfield [65] has suggested that Amycolatopsis species is the most efficient providing vanillin concentrations up to 10g/L in a fermentation period of only 36 h [88,89]. This is an excellent yield and few side products were found.

9.7.3.3 Resolution of Racemic Mixtures

Just as enzymes find utility in the resolution of racemic mixtures, so do microorganisms. They both show very strong chiral preferences in their activities. The

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resolution of menthol racemates is an example of commercial application of this approach [68].

In this example, synthetic D,L-menthol racemate is initially chemically esterified with acetate, formate, propionate, myristate, benzoate, or succinate. The ester is then selectively hydrolyzed by steriospecific microbial enzymes to produce L-menthol that is readily isolated from the D-menthol ester. The low water solubility of the menthyl esters and L-menthol has made this process particularly suitable to non-aqueous systems. Omata et al. [90] immobilized cells of *Rhodotorula minuta* in photo-cross-linked or polyurethane resin gels and used a water saturated hexane eluant to obtain a product of 100% optical purity. The immobilized cells had an estimated half-life of 55 to 63 days in this solvent.

9.7.4 Economics of Biotechnology

It is generally impossible for biotechnologically produced aroma chemicals to compete economically with their chemically synthesized counterparts. Synthetic aroma chemicals often sell for $5 to 50 per kg while the equivalent chemicals produced via biotechnology generally cost 10 to 100 times more. The cost of fermentation, low yields and subsequent recovery costs (e.g., from a fermentation broth) drive these costs up greatly. The justification for paying this premium in cost is being able to use a natural label on the product. The industry appears willing to pay this premium in many cases and thus, biotechnology continues to be applied in the flavor industry.

REFERENCES

1. Buyukpamukcu, E., Understanding cocoa flavor: where does it all come from?, *Chem. Rev.,* 11, 4, p. 8, 2002.
2. Chen, C.Q., E. Robbins, Chocolate flavor via the Maillard reaction, in *Caffeinated Beverages*. T.H. Parliment, C.-T. Ho, P. Schieberle, Eds., Amer. Chem. Soc., Washington, D.C., 2000, p. 286.
3. Martin, R.A., Jr., Chocolate. *Advan. Food Res.,* 1987, 31, p. 211.
4. Ikrawan, Y., S. Chaiseri, O. Vungdeethum, Effect of fermentation time on pyrazine concentration of Thai Forasero beans. *Kasetsart J. Natural Sci.,* 1997. 31, 4, p. 479.
5. Mermet, G., E. Cros, G. Georges, Preliminary study to optimize cocoa roasting parameters: aroma precursor consumption, pyrazine development, organoleptic quality. *Cafe Cacao,* 1992. 36, 4, p. 285.
6. Cros, E., Effect of roasting parameters on the development of cocoa aroma. *Ind. Aliment. Agric.,* 1993. 110, 7-8, p. 535.
7. Hashim, L., Flavor development of cocoa during roasting, in *Caffeinated Beverages*. T.H. Parliment, C.-T. Ho, P. Schieberle, Eds., Amer. Chem. Soc., Washington, D.C., 2000. p. 276.
8. Allerton, J., Chocolate and cocoa products, in *Encyclopedia of Food Technology*, A.H. Johnson, M.S. Peterson, Eds., AVI Pub. Co., Westport, 1974, p. 195.
9. Minifie, B.W., *Chocolate, Cocoa and Confectionery: Science and Technology,* 3rd ed. Van Nostrand Reinhold, New York, 1989, p. 904.
10. Beckett, S.T., *Industrial Chocolate Manufacture and Use,* 2nd ed., Blackie Academ. Prof., New York, 1994, p. 408.

11. Schnermann, P., P. Schieberle, Evaluation of key odorants in milk chocolate and cocoa mass by aroma extract dilution analyses, *J. Agric. Food Chem.*, 45, 3, pp. 867–872, 1997.
12. Schieberle, P., P. Pfnuer, Characterization of key odorants in chocolate, in *Flavor Chemistry: Thirty Years of Progress*, R. Teranishi, E.L. Wick, I. Hornstein, Eds., Kluwer Acad. Publ., New York, 1999, p. 147.
13. Hodge, J.E., F.D. Mills, B.E. Fisher, Compounds of browned flavour derived from sugars-amine reactions, *Cereal Sci. Today*, 17, p. 34, 1972.
14. Melton, S.L., Current status of meat flavor, in *Quality Attributes of Muscle Foods*, Y.L. Xiong, C-T Ho, F. Shahidi, Eds., Kluwer Academ. Publ., New York, 1999, p. 115.
15. Mottram, D.S., Flavor formation in meat and meat products: a review, *Food Chem.*, 62, 4, pp. 415–424, 1998.
16. Pearson, A.M., J.I. Gray, C.P. Brennand, Species-specific flavors and odors [of meat], in *Quality Attributes and Their Measurement in Meat, Poultry and Fish Products*, A.M. Pearson, T.R. Dutson, Eds., Blackie Academ., London, 1994, pp. 222–49.
17. Reineccius, G., Flavor and aroma chemistry [of meat], in *Quality Attributes and Their Measurement in Meat, Poultry and Fish Products*, A.M. Pearson, T.R. Dutson, Eds., Blackie Academ., London, 1994. p. 184–201.
18. Farmer, L.J., Meat flavor, in *Chem. Muscle-Based Foods* (Special Publication), Royal Soc. Chem., 106, pp. 169–82, 1992.
19. Nagodawithana, T., *Savory Flavors*, Esteekay Assoc., Milwaukee, 1995, p. 468.
20. Aaslyng, M.D., 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, 12, pp. 5225–5231, 1998.
21. Wu, Y.-F.G., K.R. Cadwallader, Characterization of the aroma of a meat-like process flavoring from soybean-based enzyme-hydrolyzed vegetable protein, *J. Agric. Food Chem.*, 50, 10, p. 2900, 2002.
22. Gasser, U. and W. Grosch, Identification of volatile flavor compounds with high aroma values from cooked beef, *Zeitschrift Lebens. Untersuch. Forsch.*, 186, 6, p. 489, 1988.
23. Gasser, U., W. Grosch, Primary odorants of chicken broth: a comparative study with meat broths from cow and ox, *Zeitschrift Lebens. Untersuch. Forsch*, 190, 3, p. 3, 1990.
24. Gasser, U., W. Grosch, Aroma extract dilution analysis of commercial meat flavorings, *Zeitschrift Lebens. Untersuch. Forsch*, 190, 6, p. 511, 1990.
25. Grosch, W., G. Zeiler-Hilgart, Formation of meat-like flavor compounds, in *Flavor Precursors*, R. Teranishi, G.R. Takeoka, M. Güntert, Eds., Amer. Chem. Soc., Washington, D.C., 1992. p. 183.
26. Cerny, C., W. Grosch, Evaluation of potent odorants in roasted beef by aroma-extract dilution analysis, *Zeitschrift Lebens. Untersuch. Forsch*, 194, 4, p. 322, 1992.
27. Cerny, C., W. Grosch, Quantification of character-impact odor compounds of roasted beef, *Zeitschrift Lebens. Untersuch. Forsch*, 196, 5, p. 417, 1993.
28. Grosch, W., et al., Studies on the formation of odorants contributing to meat flavors, in *Progress Flavour Precursor Studies*, P. Schreier, P. Winterhalter, Eds., Allured Publ., Carol Stream, 1993, p. 329.
29. Cerny, C., W. Grosch, Precursors of ethyldimethylpyrazine isomers and 2,3-diethyl-5-methylpyrazine in roasted beef, *Zeitschrift Lebens. Untersuch. Forsch*, 198, 3, p. 210, 1994.
30. Kerler, J., W. Grosch, Character impact odorants of boiled chicken: changes during refrigerated storage and reheating, *Zeitschrift Lebens. Untersuch. Forsch.*, 205, 3, p. 232, 1997.

31. Kerscher, R., W. Grosch, Comparative evaluation of potent odorants of boiled beef by aroma extract dilution and concentration analysis, *Zeitschrift Lebens. Untersuch. Forsch.* 204, 1, p. 3, 1997.
32. Kerscher, R., W. Grosch, Comparison of the aromas of cooked beef, pork and chicken, in *Frontiers of Flavour Science*, P. Schieberle, K.H. Engel, Eds., Deutsche Forschung. Lebensmit., Garsching, 2000, p. 17.
33. Wasserman, A.E., N. Gray, Meat flavor. I. Fractionation of water-soluble flavor precursors of beef, *J. Food Sci.,* 1965. 30, 5, p. 801.
34. Zaika, L.L. Meat flavour: method for rapid preparation of the water-soluble low molecular weight fraction of meat tissue extracts, *J. Agric. Food Chem.*, 17, 4, 893, 1969.
35. Wasserman, A.E., A.M. Spinelli, Effect of some water-soluble components on aroma of heated adipose tissue, *J. Agric. Food Chem.*, 20, 2, p. 171, 1972.
36. Manley, C.H., Flavor Interactions Workshop, IFT sponsored, Orlando, FL, 2003.
37. Blake, A., The world of meat flavours, *Food Manuf.*, 57, 9, p. 65, 1982.
38. Prendergast, K., Protein hydrolysis — a review, *Food Trade Rev.*, 44, p. 14, 1974.
39. Roozen, J.P., The bitterness of protein hydrolysates, in *Progress in Flavour Research*, J. Adda, Ed., Applied Sci. Publ., London, 1979, p. 321.
40. Sakho, M., Enzymatic maceration: effects on volatile components of mango pulp, *J. Food Sci.*, 63, 6, p. 975, 1998.
41. Winterhalter P., G.K. Skouroumounis, Glycoconjugated aroma compounds: occurrence, role and biotechnological transformation, *Adv. Biochemical Engin./Biotechnol.*, 55, p. 73, 1997.
42. Berger, R., G., U. Krings, H. Zorn, Biotechnological flavour generation, in *Food Flavour Technology*, A.J. Taylor, Ed., Sheffield Academ. Press, Sheffield, U.K., 2002, pp. 60–104.
43. Freund, P., Flavor Workshop II: Reaction Flavors, Encapsulation and Analysis, University of Minnesota, St. Paul, September, 2003.
44. Kilcawley, K.N., M.G. Wilkinson, P.F. Fox, Enzyme-modified cheese, *Int. Dairy J.*, 8, p. 1, 1998.
45. Freund, P.R., Cheese flavours ripen into maturity, *Int. Food Ingred.*, 5, p. 35, 1995.
46. Freund, P.R., Speciality milks and milk derived flavors in confections, *Manuf. Confect.*, 75, 6, p. 80, 1995.
47. Klein, N., S. Lortal, Attenuated starters: an efficient means to influence cheese ripening — a review, *Int. Dairy J.*, 9, p. 751, 1999.
48. West, S., Flavour production with enzymes, in *Industrial Enzymology*, T. Godfrey, S. West, Eds., Macmillan Publ., London, 1996, p. 211.
49. Andres, C., Butter flavor variations meet specific usage demands, *Food Process,* 1981. 42, 5, p. 62.
50. Farnham, J.G., R. Jordan, H. Saal, Spray/mat effectively dries tough enzyme modified butter-oil, cheese, *Food Process*, 42, 3, p. 104, 1981.
51. Hori, S., H. Shimazono, Intensification and supplementation of the flavor of dairy products, GB Patent #1075149, 1967.
52. Piatkiewicz, A.E.A., Selection and mutation of *Streptococcus diacetylactis* for butter production, in *Advances in Biotechnology Vol. II. Fuels, Chemicals, Foods and Waste Treatment*, A. R. Liss, New York, 1981, p. 491.
53. Olson, N.E.A., Microencapsulation of multi-enzyme systems, in *Advances in Biotechnology: Vol. III. Fermentation Products*, A. R. Liss, New York, 1981, p. 361.
54. Kilara, A., Enzyme modified lipid food ingredients, *Process Biochem.*, 20, p. 35, 1985.
55. Birschbach, P., Origins of lipases and their action characteristics, in Bulletin 294, *Int. Dairy Fed.*, Brussels, p. 7, 1994.

56. Birschbach, P., Pregastric lipases, in Bulletin 269, *Int. Dairy Fed.*, Brussels, p. 36, 1992.
57. De Greyt, W., A. Huyghebaert, Lipase catalyzed modification of milk fat, *Lipid Technol.*, 7, p. 10, 1995.
58. Kwak, H.S., I.J. Joen, S.K. Perng, Statistical patterns of lipase activities on the release of short chain fatty acids in Cheddar cheese slurries, *J. Food Sci.*, 54, p. 1559, 1989.
59. Kristofferson, T., E.M., J.A.G. Mikolejeik, Cheddar cheese flavor: 4. Directed and accelerated ripening process, *J. Dairy Sci.*, 50, p. 292, 1967.
60. Harvest Fields, *Vinegar Types, Methods of Making Vinegar & Recipes,* The Harvest Group, http://www.harvestfields.netfirms.com, accessed 04/19/05.
61. Napoleon Company, *Balsamic vinegar*, http://www.napoleon-co.com, accessed 9/13/04.
62. Zingermans Co., *The Truth About Balsamic Vinegar*, http://www.zingermans.com/Category.pasp?Category=balsamic%5Fvinegar, accessed 04/19/05.
63. Henry, B.S., The Production, Properties and Applications of Inactive Yeast Powders and Extracts, Proc. Symp. British Soc. Flav., London, June, 1982.
64. I.O.F.I. (International Organization of the Flavor Industry) http://www.iofi.org, accessed 04/19/05.
65. Gatfield, I.L., Biotechnological production of natural flavor materials, in *Flavor Chemistry: Thirty Years of Progress*, R. Teranishi, E.L. Wick, I. Hornstein, Eds., Kluwer Acad. Publ., New York, 1999, p. 211.
66. Parliment, T.H., R. Croteau, Eds., *Biogeneration of Aromas*, Amer. Chem. Soc., Washington, D.C., 1986, p. 397.
67. Berger, R.G., *Aroma Biotechnology*, Springer Verlag, Berlin, 1995, p. 240.
68. Schreier, P., Enzymes and flavour biotechnology, in *Advances in Biochemical Engineering*, R.G. Berger, Ed., Springer Verlag, Berlin, 1997, p. 51.
69. Welsch, F.W., W.D. Murray, R.E. Williams, Microbiological and enzymatic production of flavor and fragrance chemicals, *Crit. Rev. Biotechnol.*, 9, 2, p. 105, 1989.
70. Janssens, L., H.L. De Pooter, N. Schamp, E.J. Vandamme, Production of flavours by microorganisms, *Proc. Biochem.*, 27, p. 195, 1992.
71. Gatfield, I.L., The enzymatic synthesis of esters in non-aqueous systems, *Ann. N.Y. Acad. Sci.*, 436, p. 569, 1984.
72. Gatfield, I.L., The enzymatic synthesis of esters in non-aqueous systems, *Lebensm. Wiss. Technol.*, 19, p. 87, 1986.
73. Armstrong, D.W., B. Gillies, H. Yamazaki, Natural flavors by biotechnological processing, in *Flavor Chemistry: Trends and Developments*, R. Teranishi, R.G. Buttery, F. Shahidi, Eds., Amer. Chem. Soc., Washington, D.C., 1989, p. 105.
74. Duff, S.J.B., W.D. Murray, Oxidation of benzyl alcohol by whole cells of *Pichia pastoris* and by alcohol oxidase in aqueous and nonaqueous reaction media, *Biotechnol. Bioeng.*, 34, p. 153, 1989.
75. Gatfield, I., T. Sand, Enzymic production of esters and lactones, U.S. Patent 4451565, 1982.
76. Whitehead, I.M., B.L. Muller, C. Dean, Industrial use of soybean lipoxygenase for the production of natural green note flavor compounds, in *Bioflavour 95*, P. Étiévant, P. Schreier, Eds., Institut. National de la Recherche Agronomique, Paris, 1995, p. 339.
77. Candella, C., Personal communication, Robertet Flavors, S.A., Grasse, France, 1995.
78. Shimada, Y., et al., Enzymatic synthesis of L-menthyl esters in organic solvent-free system, *J. Amer. Oil Chem. Soc.*, 76, 10, p. 1139, 1999.
79. Hilmer, J.M., U. Bornscheuer, R. Schmidt, S. Vorlova, I.L. Gatfield, Process for the preparation of L-menthol, U.S. Patent 6706500, 2004.
80. Roy, A. Chemoenzymatic synthesis of homochiral (R)- and (S)-karahanaenol from (R)-limonene, *J. Agric. Food Chem.*, 47, 12, p. 5209, 1999.

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U.S. Patent No. 11,013,250

81. McIver, R.C., *Factors Influencing the Synthesis of Methoxy Alkyl Pyrazines by Pseudomonas Perolens,* Ph.D. thesis, University of Minnesota, St. Paul, p. 88, 1986.
82. Kuila, R.K., B. Ranganathan, Ultraviolet light-induced mutants of *Streptococcus lactis* subspecies diacetylactis with enhanced acid- or flavor-producing abilities, *J. Dairy Sci.*, 61, 4, p. 379, 1978.
83. Farbood, M., B.J. Willis, Production of γ-Decalactone, U.S. Patent 4560656, 1985.
84. Van der Schaft, P.H., N. Ter Burg, S. Van den Bosch, A.M. Cohen, Microbial production of natural δ-decalactone and δ-dodecalactone from the corresponding α, β-unsaturated lactones in massoi bark oil, *Appl. Microbiol. Biotechnol.*, 36, 6, p. 712, 1992.
85. Dziezak, J.D., Biotechnology and flavor development: an industrial research perspective, *Food Technol.*, 40, 4, p. 108, 1986.
86. Sharpell, F., C. Stegmann, Developments of fermentation media for the production of butyric acid, in *Advances in Biotechnology*, M. Moo-Young, Ed., Pergamon Press, Toronto, 1981, p. 71.
87. Hopp, R., P, J. Rabenhorst, Process for the preparation of vanillin and microorganisms suitable therefor. U.S. Patent 6133003, 2000.
88. Priefert, H., J. Rabenhorst, A. Steinbuchel, Biotechnological production of vanillin, *Appl. Microbiol. Biotechnol.*, 56, 3–4, p. 296, 2001.
89. Rabenhorst, J., R. Hopp, Process for the preparation of vanillin, U.S. Patent 5017388, 1991.
90. Omata, T., Stereoselective hydrolysis of D,L menthyl succinate by gel-entrapped *Rhodotorula minuta* var. texensis in organic solvent, *Appl. Microbiol. Biotechnol*, 11, p. 199, 1981.
91. Mathewson, P.R. *Enzymes*, Eagan Press, St. Paul, 2004, p. 109.
92. Richardson, T., D.B. Hyslop, Enzymes, in *Food Chemistry,* 2nd ed., O.R. Fennema, Ed., Dekker, New York, 1985, p. 371.