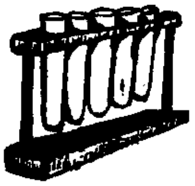

Processing and Biotechnology as a Source of Flavors

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The most interesting and most rapidly growing area in the flavor industry today deals with creation of flavoring and flavoring components from processing and biotechnology.

Historically the flavor industry developed by the use of naturally derived materials from botanicals (herbs and spices), other extractives (vegetable, yeast, etc.) and hydrolysates. With the development of the science of synthetic organic chemistry, many chemicals resembling those found in nature were created and used in the fabrication of flavors.

It was not until the mid 20th century with the development of sophisticated analytical equipment that scientists found that many of those synthetic chemicals causing certain aromas were identical to chemicals found in the natural products. Certainly the natural product chemist during the 1960s and 1970s isolated and identified many components that were then synthesized and used as GRAS (Generally Regarded As Safe) chemicals to build artificial flavors.

The development of the GRAS concept was, of course, a significant milestone for the U.S. flavor industry. The concept was developed in the late 1950s by the industry (specifically by Dr. Ben Oser and Dr. Richard Hall) to take advantage of the 1958 Food Additive Regulations.

That then, in a brief review, is the history of the industry from the early times to the mid 1970s, a business based on natural extractives and synthetic chemicals producing a variety of flavorings. Then entered the natural products chemist, biochemist and microbiologist and, to some degree, marketing with its concept of natural flavors.

The need for complex aroma blends, particularly for the creation of meat, chocolate and cheese flavors was beginning to become intense in the late 1970s. The technical efforts to create these flavors from natural extracts and/or synthetic chemicals left something to be desired. It was the natural products chemist who started to define what was chemically going on in the natural system, i.e., why certain meats develop the aroma profile that they do upon cooking.

The use of the scientific results of those studies gave rise to new directions for the industry in producing flavors. Processed flavoring technologies were used to create meat, cheese and chocolate flavors. For years hydrolyzed vegetable proteins had been used as bases for meat flavorings, and the use of controlled enzyme action created autolyzed yeast for cheese (EMC) and meat flavor. Further enzyme-modified cheese from green cheese solids contributed to the de-

Sulfur Source	Carbonyl Compound	Medium	Type Meat Flavor
Cysteine	Hexose	Water	Boiled
Thiamine	Pentose	Fat	Roasted
Hydrogen	Aldehyde		
Sulfide	Furanone	Food	Boiled

Figure 1. Preparation of Meat Flavors from Precursors

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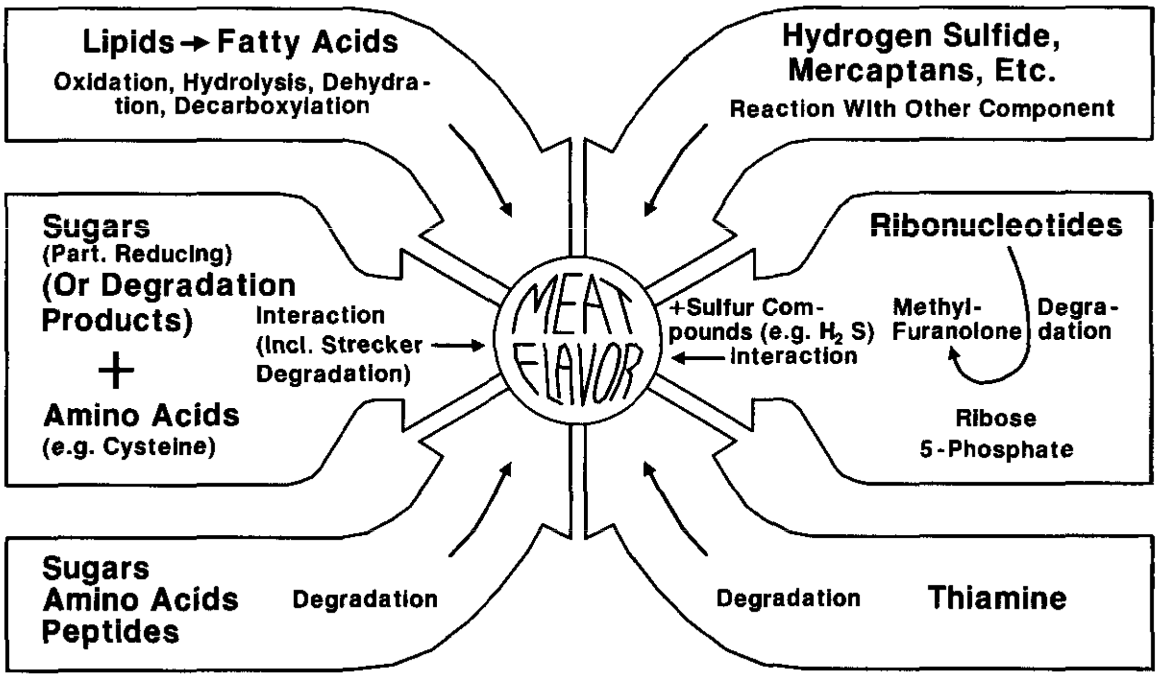

Figure 2. Production of Meat-like Aromas

velopment of cheese flavor along with modified milk fats. Most recently the use of microorganism (fermentations) has contributed other useful materials for the flavorist.

Process Flavors

By the mid 1970s, a great deal was understood about the chemistry of roasting and/or cooking of foods. Natural precursor materials that resulted in meat aroma were identified and model systems were developed to produce those aromas (see figure 1). The field of process flavor (particularly thermally processed flavors) was firmly established.

Use of Maillard reactions—reducing sugars reacting with amino acids via the Strecker degradation pathway—was one route which was well-studied and known for many years. Pyrolysis products of sugars, amino acids and peptides was another mechanism. Controlled oxidation of lipids and fatty acids produced meat characterizing aromas (chicken, for example). The degradation of vitamins such as vitamin B1 (thiamine) produced strong meat-like aromas and so do other sulfur-containing materials like hydrogen sulfide or mercaptans. Even the nucleotides of the plants and animals were found to contribute to meat aroma when heated alone or with sulfur-containing chemicals (see figure 2).

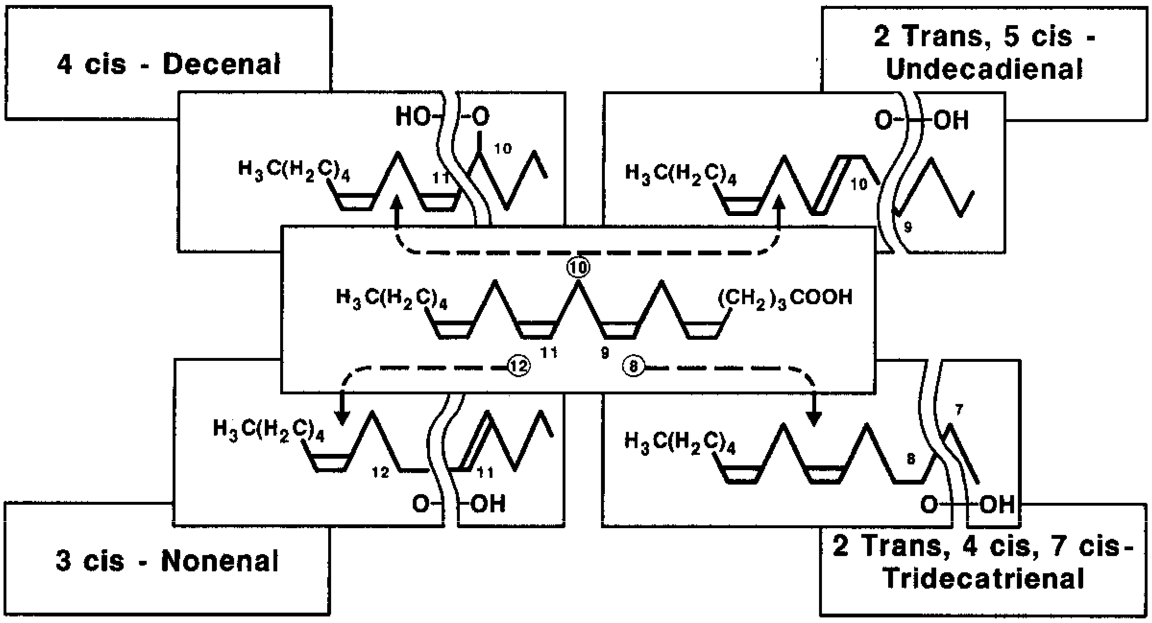

Figure 3. Flavors from Controlled Oxidation

Let me give the natural product chemists a few examples of this area of process chemistry. Chicken fat contains a high amount of unsaturated fatty acid; the complete oxidation of this material leads to rancidity and products of negative value; but by controlling the oxidation—Dienal and Trienal (aldehydes with two or three sites of unsaturation)—useful flavors may be formed (see figure 3). These materials are strongly reminiscent of a boiled or cooked chicken. Is there a science and, yet, an art to it? Yes, but that is mainly the proprietary knowledge of the industry. Remember the fine line between rancidity and good chicken flavor is drawn by reaction control.

Ribonucleotides, (see figure 4) so common in meat and yeast, are useful in producing a hydroxy methyl furanone compound which, when combined with sulfur, gives rise to many chemicals—all with interesting meaty-like aromas. The exact control and mixture of ingredients, of course, are protected by patents or remain a trade secret of the producing company. Can it be done by mixing synthetic components? Yes, but the components were actually first isolated from those process flavors or from roasted meat or yeast. With all these mercaptan and sulfur compounds, you can gather that control and chemical knowledge are most important—or end up with a very stinky mess. Then one man's stink is another's cherished flavor!

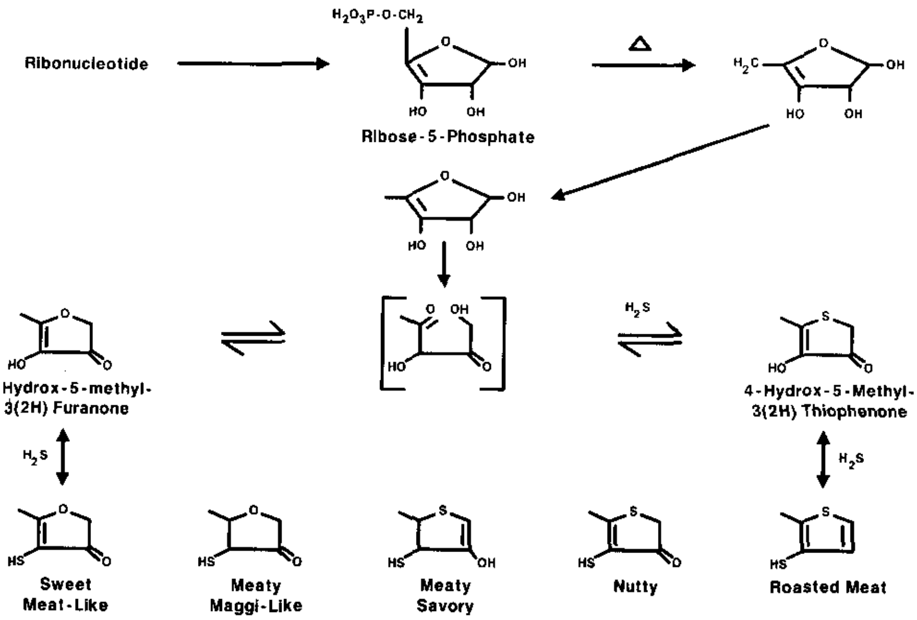

Figure 4. Meat-like Aromas from Ribonucleotides

Enzymes

The biochemists have had their day in the use of enzymes to create basic flavors. Starting with autolyzed yeast extracts (AYE) and enzyme modified cheese, the biochemists' use of enzymes for specific flavor creation has increased dramatically in the last decade. One interest area has been the enzyme modification of milk fat for flavor use. It is well known that milk can be turned into interesting products through the control of lipolytic rancidity. Products such as cultured buttermilk and cheese were the results of such processing. Once again the exact control of any process will govern the quality and usefulness of the final product. Lane and Hammer's work in the 1930s indicated the chemical process of lipolytic rancidity and the enzymes involved. They were able to utilize artificially manipulated lypolysis for the manufacture of blue cheese. Lypolysis has been defined as the enzyme-catalyzed hydrolytic cleavage of triglycerides resulting in the release of free fatty acids which, in most cases, often reduces the appeal of many foods. The spontaneous lipolysis by endogenous enzymes has plagued many sectors of the food processing industry for some time. Lipolysis is brought about by two major groups of enzymes—lipases and esterases—both being involved in fat metabolism or catabolism. The most used enzyme today for this purpose is the pharageal-derived esterase.

The major effect of lipolysis has been to allow one to create useful free fatty acid profiles. Historically we can see that certain changes in fatty acids resulted in what was considered more desirable flavor profiles. Lipolytic rancidity in farm separated cream was a common occurrence, and butter produced from it generally was of a coarse, pungent flavor due to release of short chain fatty acids. This type of flavor was not suited for table use, but did find use by cooks for preparing baked goods. Certain types of cheese, notably Italian and mold-ripened (roquefort, blue) cheeses normally exhibit flavor notes associated with mid-range fatty acids.

The method can thereby be used to create products that are useful in fortifying chocolate products, butter flavor, milk and cream flavors and, of course, cheese flavors. A great amount of enzyme modified cheese is being used to fortify processed cheese, cheese powders for snack products and in ultra high temperature (UHT) cheese sauces.

Enzymes are finding uses in other areas, for example in cheddar cheese flavors, enzyme meat digests, yeast autolysate processing, onion flavor (excellent work was done by IFF in late 1960s) and in tea flavor formation by the use of tannase.

Biotechnology

Now let me turn your attention to biochemistry and microbiology, two recent newcomers to the field of the flavor industry. Needless to say "biotechnology," the "new" high tech term, has traditionally been applied in the preparation of a number of food products including many which have unique flavors (we used to call it fermentation). The brewing of beer, the ripening of cheese, the "Koji"—brewing of soy sauce—have all used one facet of the science of biochemistry and/or microbiology. More recently, biotechnology-based knowledge has been used in the synthesis of antibiotics, amino acids, 5'nucleotides and organic acids. These materials are produced by fermentation. Even high volumes of food ingredients such as glucose and high fructose syrups have been produced by fermentation.

If the technologies of thermal processing or enzymes were methods needing constant control, then the technology of bioconversions is even more demanding. Enzymes from microorganisms are, generally, rather labile so that very precise reaction conditions must be established and maintained if the biocatalyst is to render the proper products.

Let us explore some of the potentials that biotechnology may offer.

The creation of 5-keto-gluconic acid from glucose is a known bacterial oxidation (see figure 5). The novelty of creating such a material is that 5-keto-gluconic acid upon heating forms 4-hy-

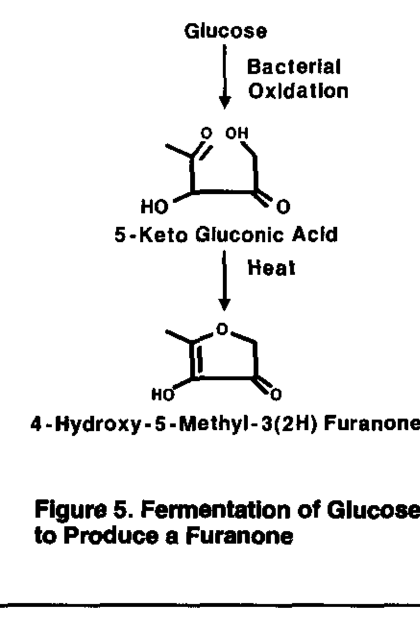

Figure 5. Fermentation of Glucose to Produce a Furanone

droxy-5-methyl 3(2H) furanone which is a major precursor material in meat flavor development. This illustrates a very convenient route to an important and expensive flavor component, made possible by the selective microbial oxidation of a cheap feedstock, glucose, in combination with process flavor technology. It is the major route to creating outstanding meat flavorings.

Some of the important components in butter flavorings are acetoin, diacetyl and certain lactones. These compounds can be produced by fermentation. For example gamma-decalatone is a product of fermentation of castor oil by an organism of the candida species. Other substrate/microorganism systems are based on beta oxidation of fatty acids to produce the gamma-hydroxy decanoic acid followed by lactonisation.

Acetoin and diacetyl, again common compounds important for the flavor of dairy products, may be produced by the fermentation of pyruvate by lactobacillus plantarum in a pH-static culture.

Microorganisms for Flavor Development

- o Penicillium Decumbens—Sesquiterpines
- o Phellinus—Anisaldehyde/Methyl Acetophenone
- o Trichoderma Viride—Pentyl-6-α-Pyrone
- o Lasiodiplodia Theogbromae-B-Ionone—Oxo-4-β-Cyclohomo Geraniol
- o Septoria Nodorum/Bacillus Natto—Pyrazines
- o Sporobolomyces Odorus—Lactones

Although the identification of the proper organism is initially important to establishing a biotechnology method, in the end the optimum fermentation conditions for the process must be established to make the product at commercial scale. Examples of the importance of this can be seen from work done by Dr. Tom Montville at the Rutgers University's Food Science Department. By studying various fermentation parameters, Dr. Montville's group determined that the lactobacillus plantarum organism, frequently isolated from salted white pickled cheese, is a known producer of diacetyl and acetoin. The group worked with several variables and finally demonstrated that the diacetyl/acetoin synthesis can be stimulated up to 15-fold by the addition of 20 mM pyruvate. This type of fine tuning of the biotechnology method is absolutely neces-

As the development of flavors by thermal processing needs a clear understanding of the chemical mechanisms giving rise to the particular favored flavors profile, so too the development of flavors and flavor components by biochemistry needs the dedication of groups of basic research microbiologists to optimize and scale up fermentation.

Another example of microorganism which could be used to produce interesting flavoring materials is ceratocystis. This fungus, particularly ceratocystis variospora, has been shown to produce the monoterpenes, geraniol and citronella, two useful materials for flavor-compounding work. MIT scientists have indicated that the biosynthesis is via the mevalonic acid pathway.

Regulatory Issues

But now let us talk about the future of biotechnology—the use of genetic engineering to selectively change an organism to produce a particular material or mixture or a higher yield of a material. Maybe in the end we will have a microorganism producing a flavor mixture once produced by a mint leaf, an apple or a strawberry. Perhaps a far out idea at this time, but twenty years ago genetic engineering was not even an idea!

I am sure that the initial use of genetics will focus on the ability of microbiologists to induce genetic changes which allow for increasing yield(s) of specific chemical component(s). In the U.S. alone this is a market which is estimated to be 400 to 500 million dollars in value. Because of the natural connotation of fermentation-derived materials, a great segment of that market could be available to materials produced by this technology.

The only questions to be asked are those regarding the economics and the regulatory status of the materials produced. In a recent issue (January 1987) of *Food Drug Cosmetic Law Journal*, Stephen H. McNamara reviewed the current regulations regarding the new techniques of biotechnology. He indicates that the new technologies do present new safety concerns and believes that those products would inevitably be subjected to regulation by the federal government for the purpose of assuring safety. Mr. McNamara concludes his paper by stating:

> "To regulate food substances that are produced by modern biotechnology, FDA has stated that it intends to apply existing requirements of law and related procedures concerning added poisonous or deleterious substances, food additives, and GRAS substances. The agency does not intend to establish new requirements or procedures specifically for food substances that are developed with new techniques of biotechnology.
>
> Agency statements suggest that new food additive regulations or a new GRAS affirmation regulation may be required for a food substance that is pro-

duced by new techniques of modern biotechnology. However, legal precedents suggest that at least some of these substances may qualify as GRAS without need for the issuance of any regulation or may come within the scope of existing GRAS or food additive regulations, in which case additional agency approval prior to marketing should not be required."

If we review the total regulatory situation regarding both process flavor and biotechnology, it is clear that most people in the industry are interpreting the regulations under the Flavor Labeling Section 21 CFR 101.22 and GRAS Section 21 CFR 182 as to the legal status of these materials. It is therefore generally believed that materials produced by fermentation and/or processing are GRAS if no hazards are found and that they are natural if the starting materials are also natural.

Congressional Issues

Congressman John D. Dingell (D. Michigan), Chairman of the House Committee on Energy and Commerce, has held hearings on the subject of biotechnology and has expressed the view that additional legislation may be needed.

He has indicated that everyone wants the system to be simple and efficient and to permit growth in this new area. However, he is concerned that there will be early errors. These errors would lead to a regulatory climate which overemphasizes the hazards. He believes that the application of existing laws to the practice is far from clear and there will be tests in court. Finally he believes that the assertion that genetic manipulation is entirely safe is premature. He points to the recent revelations regarding toxic wastes, industrial chemicals and radiation and concludes that there is often no evidence of a hazard because no one had looked for it.

Economic Issues

The economic issue is a difficult one. There is, indeed, some elasticity to the price of flavors which perform better or have a favorable label (natural, for example), but I believe the upper limitations are reasonably low. If they were high, the use of naturally derived materials from fruits, etc., would be in widespread use. These natural materials lack strength and stability. High use level does help, but most food companies are not interested. Artificial flavor solves these problems but creates labeling problems. Use of fermentation-derived material offers answers; but will the price be reasonable? So the role of the microbiologist and biochemist in optimizing the processes is key to these developing industries and in creating products at affordable prices.

Future Direction

There are compelling reasons for growth and development in the area of process flavor and biotechnologically developed flavor. The industry looks upon these technologies as allowing for the creation of more natural components with, in general, a wider dynamic range of use. The genetic area offers great promise, but the degree of research investment will inhibit expansion for some time to come, particularly in the flavor industry. Breakthroughs in genetic engineering in other areas (drug research, for example) will occur before a significant use of this technology in the creation of flavoring materials. Cell culture technology will, no doubt, play a very strong part in the near future, but again major breakthroughs will be made in other industries before it is applied in the development of flavors. Those of us in the industry are excited to see the new technologies developing, but we realize that there is still a great commitment to be made.

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