THE CHEMISTRY OF FLAVOR

Flavor is no longer considered a gastronomical luxury. New methods of flavor analysis may bring us better food

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YEAR BY YEAR, more and more of the food we eat is processed — canned, frozen, dehydrated, smoked, or otherwise treated so that it can be conserved. The public is already well aware of the importance of retaining the vitamin content in stored and processed foods. But now it is beginning to demand similar progress toward making these foods more palatable.

As a result, research programs are being set up with the sole objective of studying one of the most important constituents of our daily food—flavor. Plant physiologists, agricultural scientists, food industries, nutritionists, even medical workers are engaged in this work. They mean to find out what processes are involved in food ripening, how flavor is formed, how it behaves during processing, how it stands up under storage and marketing conditions, and how it can be improved.

Actually, we know very little about flavor. Even when we try to define it, we have to resort to a description of its effects. We know it is essential to the quality of foods, and determines whether our reactions to them will be favorable or unfavorable. We know, too, that these reactions find their origin in our senses of taste, smell, touch, and even sight. In many cases the flavoring agents are of known chemical structures, giving definite taste impressions—such as salt or sugar, which influence our taste receptors. In other cases the flavoring agents are complex mixtures, such as we find in spices and in all natural flavors, and which act through both the taste and smell receptors. These substances belong to all classes of chemical compounds, and at present it can be predicted only to a limited degree what a given substance will smell or taste like —and this only by comparison with other substances, and following empirical rules.

It is not clear, for example, why substances which have a totally different structure—such as sucrose, dulcin, saccharin and 4-nitro-2-aminophenylpropylether—should have a sweet taste in common. The chemical formulae of these substances, together with their relative sweetness as compared with sucrose, are shown on the following page.

We do know that each of these unrelated molecular structures can be varied to a certain degree, while still retaining the sweet qualities of the original pattern. For the sugars the presence of two or more hydroxyl groups is largely responsible for their sweet taste, and a number of polyhydric alcohols, such as glycol, glycerol and inositol, give similar reactions. A few of these related substances with their relative sweetness are:

Sucrose (100)	$CH_2OH.\underbrace{CH.(CHOH)_3.C}-O-\underbrace{\overset{CH_2OH}{\overset{\vert}{C}}.(CHOH)_2.CH}.CH_2OH$
Ethylene glycol (130)	$CH_2OH—CH_2OH$
Glycerol (108)	$CH_2OH.—CHOH.CH_2OH$
Dl-erythritol (238)	$CH_2OH.CHOH.CHOH.CH_2OH$
Inositol (50)	$CHOH.CHOH.CHOH.CHOH.CHOH.CHOH$ (ring closed from first to last CHOH)

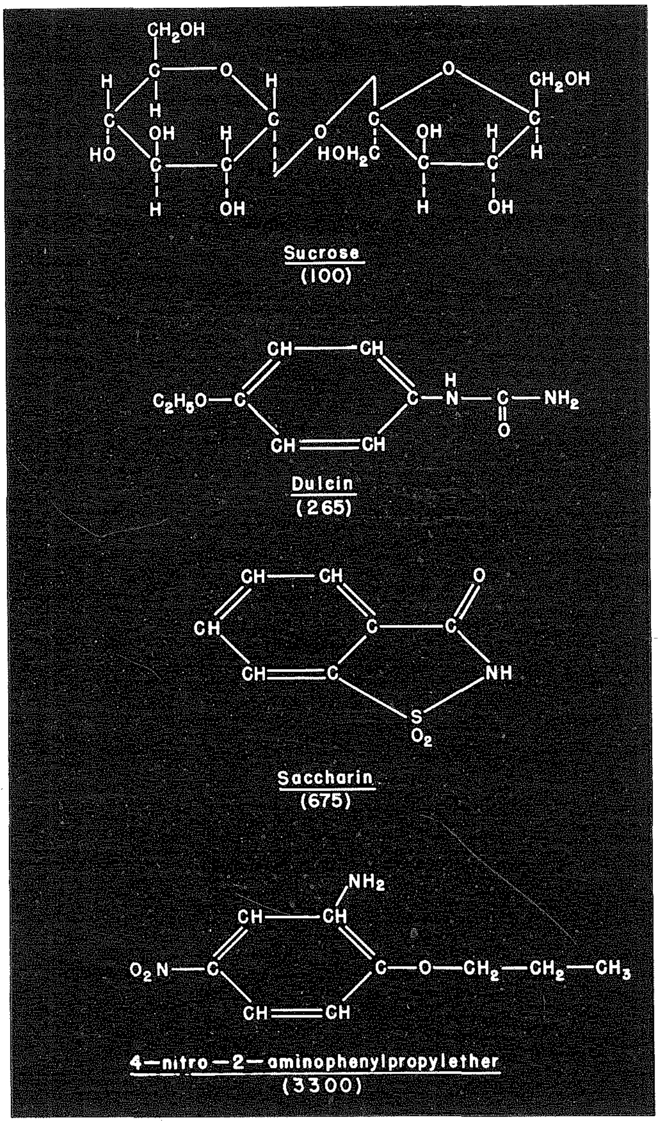
Similar observations have been made on substances giving a certain smell. While we don't know **why** a substance smells like camphor, we can empirically determine certain conditions which a compound must satisfy **before** it smells like camphor. In this case, it is generally known that organic compounds containing a carbon atom carrying several methyl groups, or groups of equal size, have camphor smell, as illustrated by the formulae on page 4.

In the case of mint flavor, we find a close relation to the camphor smell. A small change—as for example the replacement of a methyl group with a hydrogen atom—is enough to turn camphor smell into mint smell.

What makes it smell like that?

A study of the structures typical for the two types of smell shows that in all probability we are dealing with phenomena in which the shape of the molecule is of great importance. We can imagine that the shape of the molecule fits the receptors—the sensory nerve endings in the nose—and electrical impulses are set up, which travel over the sensory nerves to the brain, and give impressions corresponding to the structure of the substance.

The sensitivity of our smell and taste organs has often been used as a tool in chemical analyses. The accomplishments of these organs in a fraction of a second are quite remarkable. It often takes a chemist days to corroborate the results by strictly chemical means. For example, a trained nose can recognize nearly every member of a series of homologous alcohols, aldehydes or acids. Isomers of these compounds (molecules having the same number and kind of atoms, but in different arrangement) are more difficult to distinguish. And, in general, the variations in structure for the same type of odor and taste are so



Substances with different structures have a sweet taste in common. Figures show relative sweetness, compared with sugar.

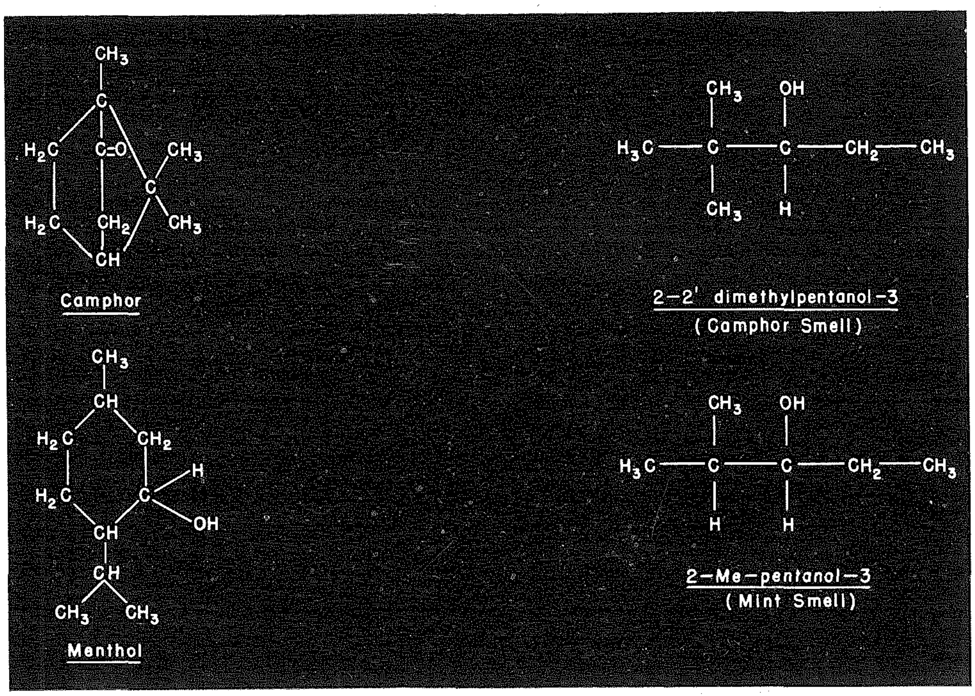
great that we cannot rely on our senses when the analysis has to be used for scientific investigations.

A more difficult task is set when mixtures of substances are present. Analysis by taste and smell breaks down completely when the substances to be recognized have not been previously isolated in pure condition. The synthetic flavor industry, for instance, can produce a more or less satisfactory reproduction of a natural flavor by skillfully combining known synthetic products. But this way of solving the flavor problem naturally reaches its limit when dealing with compounds of unknown structure. The analysis by smell and taste has, therefore, a limit set by the power of distinction of our sensory organs and by our limited knowledge of the odors of the pure compounds.

While olfactory analysis has been of great value to the composer of natural flavors, for scientific investigations such as the manner in which flavors are made by organisms, and the study of changes in flavor which occur during ripening processes, we must know the exact nature of the composition of the flavoring agents.

These considerations constitute a warning against accepting the results of smell and taste analysis, without chemical verification. Also, statements based on color reactions must be regarded with a great deal of suspicion, since closely related compounds give similar reactions. For a positive identification, the isolation of a flavor constituent or a derivative is necessary. The chemical work on flavor, therefore, consists of a process involving the isolation of the various substances contributing to the flavor, and their characterization.

The flavor substances are either volatile or non-volatile. The volatile part contains both taste and odor substances, while the non-volatile part contains taste substances only. The non-volatile substances in our food products consist mainly of sugars, fruit acids, amino acids and a number of compounds specific for the material at hand. The volatile part contains fatty acids, aldehydes, alcohols, esters, amines, and nitrogen and sulfur-containing compounds. The residue, after removal of the volatile materials, has often lost most of the flavor characteristic of that particular product. When our sense of smell is temporarily out of order,



There's no obvious relationship between menthol and camphor, but camphor and mint smells are much alike. In the simpler compounds (right) one small change — like the replacement of a methyl group with a hydrogen atom—turns one into the other.

most of our foods taste very much alike. Chemical studies, especially on fruit flavors, will therefore often be concentrated on the volatile materials.

If we compare the relative sensitivity of our senses of taste and smell, we immediately become aware of the enormous difference between the two, and of the great superiority of our odor perception. We can taste some of the bitterest substances like quinine or strychnine, in concentrations of one thousandth of a gram in a glass of water. On the other hand, we are able to detect one of the strongest-smelling substances, ethyl mercaptan (which is related to the skunk smell), in concentrations as low as one millionth of a gram in an average room. It has been calculated that our nose is about 10,000 times as sensitive as our palate. This means that, in studying the flavor constituents, we must search for odoriferous substances which occur in infinitesimal quantities. The methods to be used are therefore mostly those of the microanalyst.

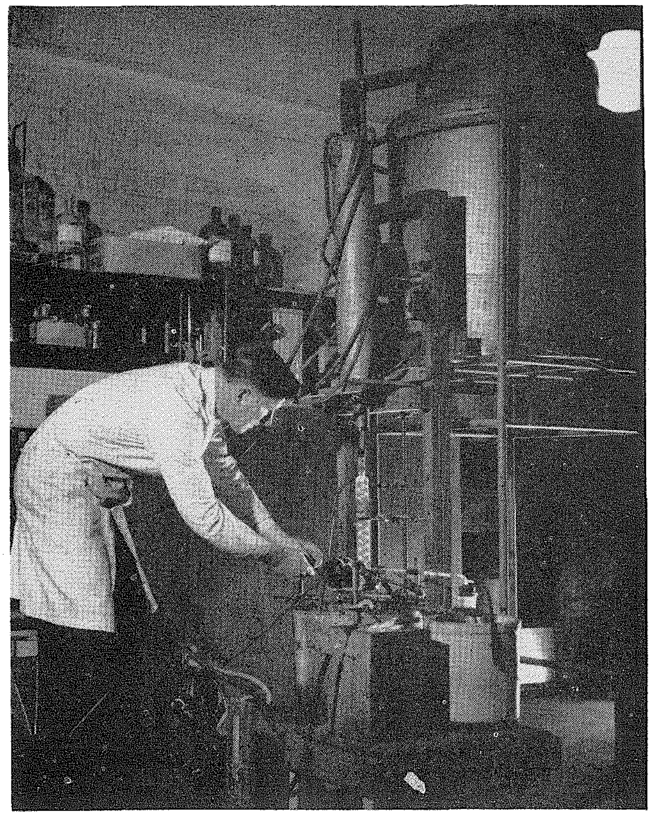
Isolating the flavor components

It is necessary to start with thousands of pounds of raw materials, however, before weighable quantities of some pure flavor components can be isolated. To obtain the volatile material we must prevent secondary changes such as are caused by heating or enzymatic reactions. A low-temperature distillation, with condensation at a lower temperature, will usually suffice to obtain all of the odoriferous substances. In other cases, solvent extraction might lead to similar results. After separation of the flavor components from large amounts of water, the individual components are separated by fractional distillation or chemical methods. The pure compounds are then characterized by the preparation of derivatives which are crystalline, and which can be compared with derivatives of known compounds of the same constitution.

These derivatives can often be used to advantage in the separation technique. When, for example, a mixture of acids is present, we can prepare the phenylphenacyl derivatives and pass the mixture through a column of adsorbent material. The different components in the mixture will be adsorbed at different heights on the column, and can be made visible in this case, since the phenylphenacyl derivatives fluoresce in ultra-violet light.

When the pure derivative of the unknown flavor substance is obtained, a comparison with the same derivative of a known substance will usually establish the identity. If the substances isolated have not been previously known, degradation methods have to be carried out to identify smaller parts of the molecule. Afterwards, the results have to be integrated to give the structure of the original compound. An example of this type of work is the analysis of pineapple flavor recently made at the California Institute of Technology.

Dr. Royal Chapman, Director of the Pineapple Research Institute, a joint enterprise of the eight Hawaiian pineapple companies, realized the importance of the exact knowledge of the pineapple flavor, for the attacking of many problems facing the industry. Such problems as the process of fruit flavor formation, and the effect of the canning process on the pineapple flavor, required for their solution fundamental knowledge of the chemical constituents of pineapple flavor. Such information could be applied to physiological and breeding work, as well as to the actual canning process. At Dr. Chapman's instigation, Caltech in 1945 undertook a research program designed to obtain such basic data.



Peeled, sliced pineapples undergo low temperature distillation, which separates flavor components from large amounts of water.

While our research was not intended as a means of obtaining a better artificial pineapple flavor, the results of our analysis would naturally lead to improved flavor formulae. For, after isolating the flavor principles, and determining their structure, it was possible to reconstruct the flavor chemically.

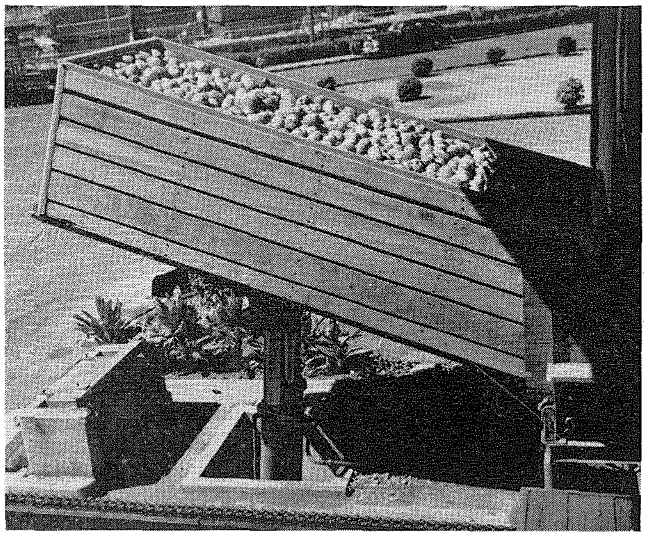
The pineapples were picked fresh at the experimental grounds in the center of the island of Oahu, and immediately brought by truck to the docks in Honolulu. They were then shipped under refrigeration, until they arrived in the harbor at San Pedro, where the Institute truck picked them up without delay. A total of approximately 6,000 pounds was used. The pineapples were peeled, cut into small pieces, and packed in large distilling flasks. (It was necessary to protect the hands with rubber gloves, because the pineapples contained an appreciable amount of protein-splitting enzyme—bromelin—which dissolves the skin rapidly, whereupon the hands become slippery, as if they had been treated with lye. Tasting experiments with fresh pineapple must also be conducted with care, since the surface of the tongue is painfully affected after some time. In canning, this enzyme is denatured).

A part of the water present in the pineapples was then drawn off under vacuum, at room temperature, to prevent any secondary decomposition. The water, which carries with it the volatile flavor components, was condensed at low temperatures (—80° C.). The condensate was redistilled, and thus the volatile flavor components were concentrated in the second distillate. Starting with several tons of material, we obtained a few ounces of volatile product which had the typical pineapple smell.

Of this quantity, ethyl alcohol and acetaldehyde formed by far the larger part. After these components had been distilled off, only a few grams were left, which contained the substances more specific for the pineapple flavor. This distillate was fractionated further by physical and chemical means.

In this fractionation process, relatively pure compounds were obtained, their properties were determined, and derivatives were made to characterize them. The majority of the compounds consisted of esters. These were hydrolyzed—chemically split into alcohols and acids through the addition of water. From the alcohol part, 3, 5 - dinitrobenzoates were prepared; from the acid part, phenylphenacyl compounds. This mixture consisted of the ethyl and methyl esters of the following acids:

Acetic acid	n-Valeric acid
Isocaproic acid	n-Caprylic acid
Hydroxyvaleric acid	Isovaleric acid



Six thousand pounds of pineapples, picked fresh, were shipped from Hawaii to Caltech for work on analysis of their flavor.

One of the fractions contained sulfur, and we were able to obtain a pure derivative by oxidation of the fraction, whereby this compound was transformed into a crystalline sulfone. Our analytical results showed that we had to deal with a compound with five carbon atoms ($C_5H_{10}SO_4$). To economize on our small amount of product, we decided to synthesize the nine possible isomers, and we found one of these identical with the natural flavor component.

Reproducing fresh pineapple flavor

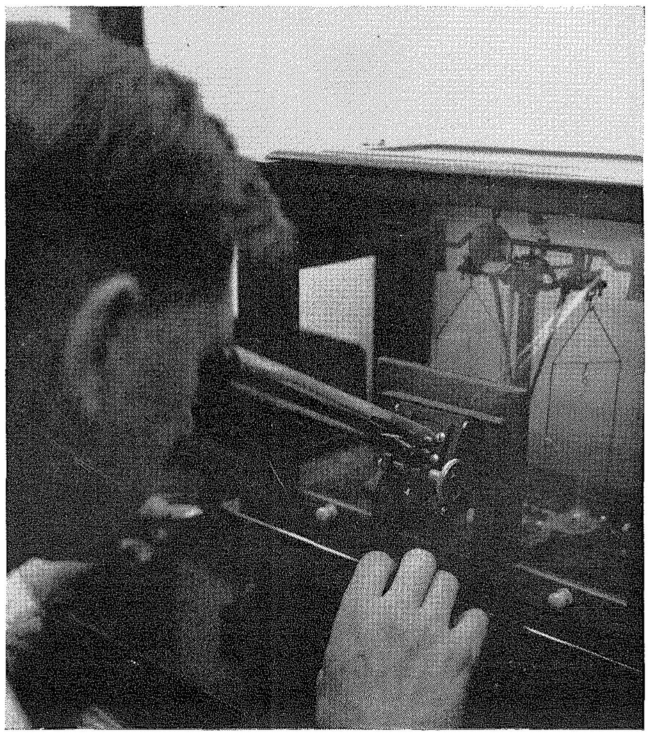
After we had analyzed about 98 per cent of the volatile components, it was interesting to see what a reconstruction of the flavor would yield. The result was a satisfactory reproduction with a fresh pineapple smell. For reproduction of the full flavor, the non-volatile substances such as sugars and different plant acids had to be added. This flavor is considerably different from that which we usually consider as typical pineapple flavor. Through the canning process some of the esters present in the fruit are hydrolyzed, and some of the fatty acids are set free, which impress upon the canned material its typical taste.

In the analysis of the flavors of natural products, the chemist tries to avoid any secondary changes in his material in order to prevent unnecessary complication of his work. However, a number of food products have been made to undergo changes for the sake of the development of their flavors. These changes may be a consequence of enzyme action, heat treatment or oxidation.

An example of the action of enzymes on the production of taste and odor is found in the onion. Chemical analysis has shown that a glucoside present in the onion is decomposed, and gives allyl sulfide and thio ethers. An example of the action of heat on a natural product is found in the production of maple flavor from maple exudate. In such a case we find not only the original components, but also their conversion products responsible for the typical maple flavor. Sugars are converted into cyclic compounds, and brown-colored products appear (caramel, coffee, toffee, etc.)

The analysis of the mixtures of chemical substances obtained through these secondary changes involves a considerable amount of work. The problem becomes a great deal more complex when the starting material is not homogeneous of origin (as it is in the pineapple, maple sap, or the onion) but rather the result of the cooperation of several organisms, as, for example, in fermented products. In such cases we must try to identify not only the contributions of the original material but also the products produced by the micro-organisms on this material.

Starting with over two tons of pineapples, Dr. Haagen-Smit got a few ounces of volatile product with typical pineapple smell.



Such an investigation is now being carried out at Caltech on wine—the result of the action of yeast on grape juice. This study, instigated by the Wine Advisory Board of California, involves separate analysis of the grape and of the wine made of it. In order to control and modify the wine flavor it is necessary to find the pathways by which the substances making up this flavor are formed. And a prerequisite for this is a knowledge of **all** the constituents of the grape, both flavor and non-flavor components, and the action of yeast upon these. Such studies are expected to be a material contribution to the direct analysis of the bouquet of wine and to the expression of its small variations in chemical terms.

Complications in condiments

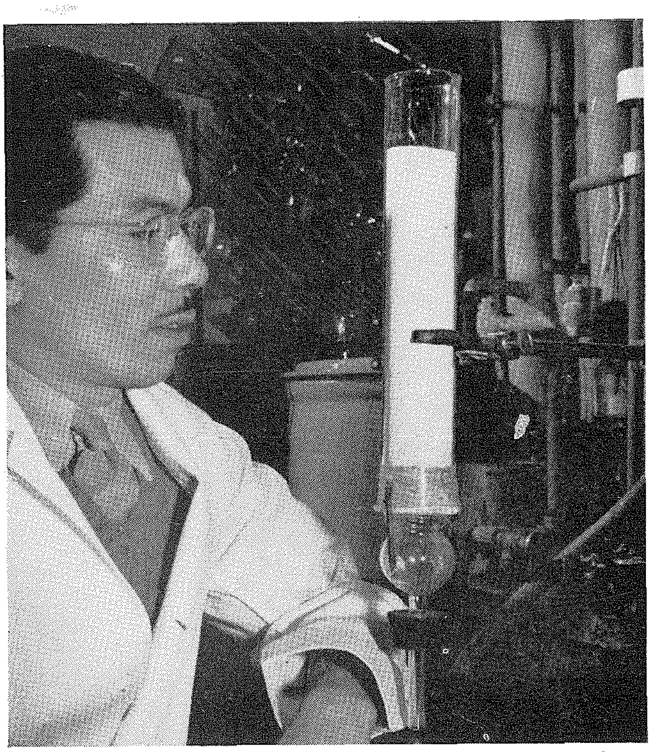
We can go still further in complicating our starting material if we combine heat treatment and growths of several fungi and bacteria. These difficulties in analysis are combined in a condiment such as soy sauce. Not only are the starting products of different origin, namely, rice and grain, but these are used as nutrient media for a number of fungi, subsequently heated, and again fermented, alternately aerobically and anaerobically (in the presence and absence of air). Many food products of great importance have such a complicated history, like bacon and other processed meat products, jellies, and preserves. With a knowledge of the chemical composition of these flavors, the effect of the different stages in processing can be accurately determined, and a better understanding of the processes involved is the result.

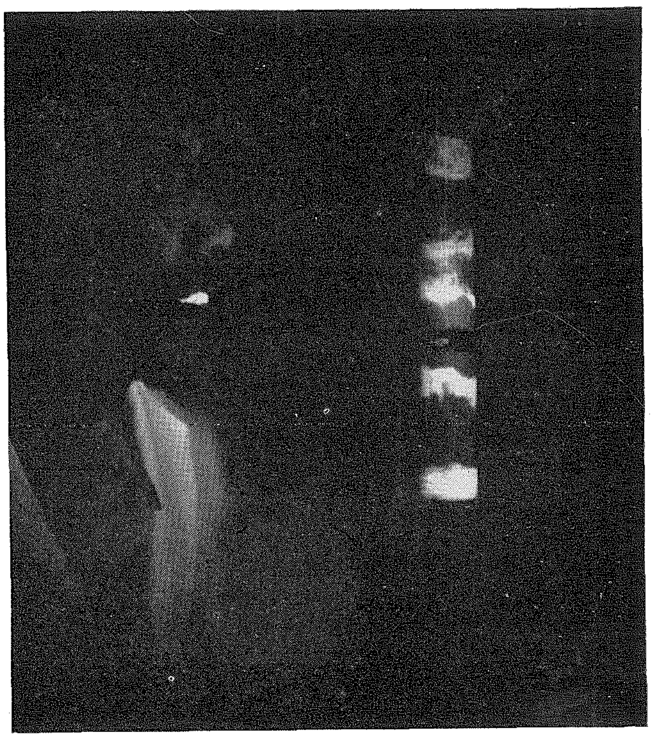
The knowledge of the composition of a flavor allows us to make predictions about the origin of the components. It is interesting to see in the case of our studies in pineapple that the esters found can all be derived quite easily from amino acids by processes involving deamination and esterification. The sulfur-containing ester, especially, shows quite clearly its precursor was an amino acid, either cysteine or methionine, and it is certainly more than a coincidence that in this fruit we find considerable quantities of a protein-splitting enzyme, and its methionine-containing activator, glutathione.

From these facts our attention is drawn to the amino acid metabolism in the fruit, rather than to the sugar metabolism, in the formation of the typical pineapple flavor. On the other hand, the general "fruitiness" of the summer fruit, caused by the presence of ethyl acetate, as compared with the bland taste of the winter fruit, links this part of the flavor to the carbohydrate metabolism. Agricultural experiments designed to improve these flavors have to modify each one of these processes. Predictions made upon the basis of these analyses can now be verified with the help of radioactive isotopes. Since a number of flavor components in the pineapple are now known, the isotope dilution method can be used to great advantage in the determination of small variations in the flavor constituents.

Among the food processing firms, it is the dehydrator who most needs the help of a systematic food analysis. In his processes, mostly based on the evaporation of water, a great many of the volatile flavoring components are removed. Dehydrated food, therefore, has received a poor reputation which can only be remedied by changed methods of dehydration and reflavoring of the dehydrated material. If the flavoring components are known, the water can be removed by a selective adsorption process in an atmosphere of the flavoring agents. In this way, odoriferous substances are prevented from escaping, and the resulting dry product retains its natural flavor.

Especially in the case of feeding large groups of people, where the free choice of food is not feasible, it has been recognized that flavor studies are of prime importance. Large organizations such as the Army and Navy, and some of the larger manufacturers of food products, have learned that in drifting farther and farther away from the use of natural foods, the processing often leads to inferior products as far as flavor is concerned. Since nutritionists have started to realize that we need palatability in addition to calories, vitamins and amino acids, flavor is no longer considered a gastronomical luxury.





After separation of flavor components of pineapples, pure compounds were characterized by preparation of crystalline derivatives. Mixture of phenylphenacyl derivatives was then passed through a column of adsorbent material (left). Different components of the mixture were adsorbed at different heights, were made visible by exposure to ultra-violet light (right).