Flavor and flavor stability of foods

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Our study on the flavor and flavor stability of fats, oils and high lipid foods can be traced to the early 1950s when I was working as a postdoctoral fellow in Dr. Kummerow's laboratory at the University of Illinois. I attempted to find out what compound or compounds are responsible for the beany and grassy flavor of soybean oil, sometimes called "reversion flavor." It was hoped that the identification of the responsible compounds would lead to the mechanism of its formation and, consequently, methods to retard or eliminate its formation.

At that time there was no gas chromatograph. Infrared spectra could be determined only with macro samples. Mass spectrometry and NMR were only fancy toys for a few specialists. We did manage, however, at that time, to determine a few simple compounds, such as n-hexanal and 2-pentenal. Now, we known such compounds occur commonly in lipid-containing foods.

By 1956, when I was working at Swift and Company, gas chromatography first became available in the United States. I remember the late Dr. Karl Mattil, who was my boss at the time, one day handed me a big roll of chart paper containing numerous gas chromatograms of the condensate collected in the cold traps during deodorization of soybean oil. It was done by an expert in gas chromatography at that time. Since the deodorization trap condensed all kinds of thermal and oxidative decomposition products of the soybean oil, and since gas chromatography was most useful for fractionation, rather than identification, the big roll of gas chromatograms served no purpose in solving the problem.

The pursuit of the compounds which are responsible for the reversion flavor in soybean oil was continued at A.E. Staley Company in 1957. I got one of the first models of gas chromatographs and used it on the volatile flavor compounds isolated from a reverted soybean oil. I was fortunate to have the cooperation of a brilliant analytical chemist, Kenneth Brobst, and Dr. Han Tai who, I believe, was one of the first to use micro infrared spectrometry to identify gas chromatographic fractions. We indentified a few aldehydes, ketones, and esters, but failed to identify the key compounds.

It was not until 1960, when I was at Rutgers University, that we could study the reversion flavor with a satisfactory methodology. An outstanding graduate student, Thomas H. Smouse, who many of you know is now a well established member of our Society, supported by a research grant from the National Soybean Processors' Association, identified 71 compounds in a reverted, but not yet rancid soybean oil. With some luck and the cooperation of other graduate students in my group at that time, he found that 2-n-pentyl furan might be very important to the reversion flavor.

We found that the addition of 2 ppu of 2-n-pentyl furan to any freshly deodorized, bland oil, whether it was cottonseed or corn oil, made the oil taste like reverted soybean oil with a beany and gassy flavor. We, therefore, concluded that 2-n-pentyl furan is primarily responsible for the reversion flavor of soybean oil. Should we have stopped here, we might have misled ourselves into believing that we had solved the mystery of the reversion flavor of soybean oil. Fortunately, we continued to pursue this interesting problem.

We proposed the mechanism of the formation of 2-n-pentyl furan and postulated that the precursor of this compound was linoleic acid (Fig. 1). Two brilliant Japanese researchers in my laboratory at Rutgers at that time, Akio Kato and Masahiko Higuchi, confirmed by well designed experiments that linoleic acid, indeed, could produce 2-n-pentyl furan by autoxidation at room temperature under diffused daylight.

An examination of the fatty acid composition of some of the commonly used vegetable oils (Table I) immediately poses a question. If the reversion flavor of soybean oil is due to 2-n-pentyl furan and, if 2-n-pentyl furan is produced by the autoxidation of linoleic acid, then other oils, such as cottonseed, corn or sunflower, which are also rich in linoleic acid, should also develop the beany and grassy reversion flavor. But they do not.

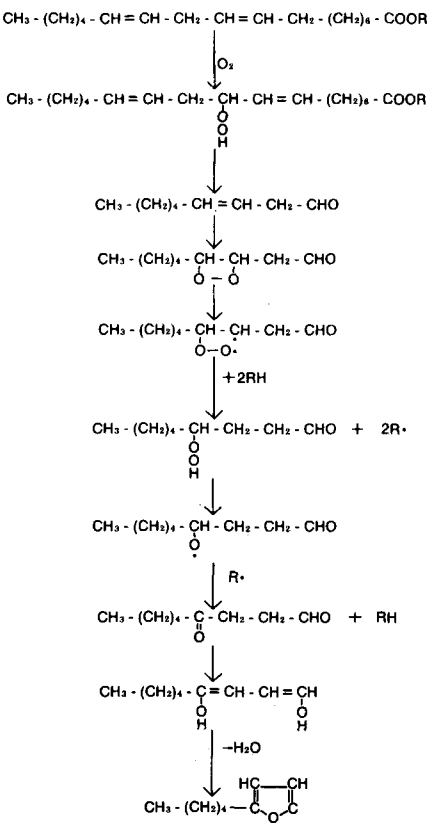


FIG. 1. Mechanism of the formation of 2-pentyl furan.

A reception address for the Lipid Chemistry Award, American Oil Chemists' Society, San Francisco, California, May 2, 1979.

TABLE I

Fatty Acid Composition of Common Vegetable Oils

	Saturated	C_{18}, 1=	C_{18}, 2=	C_{18}, 3=
Soybean oil	15	24	54	7
Cottonseed oil	26	22	52	Trace
Corn oil	15	29	56	Trace
Sunflower oil (southern)	10	37	52	Trace
Sunflower oil (northern)	10	19	70	Trace

The fatty acid which is unique to soybean oil is the 7% of linolenic acid that is absent in the other so-called non-reverting oils.

In 1977, an outstanding organic chemist, Chi-Tang Ho, joined my group as an assistant professor at Rutgers University. We proposed that if linolenic acid is unique to soybean oil, then we must examine what compound it will produce by the same mechanism as the formation of 2-n-pentyl furan from linoleic acid. We concluded that the linolenic acid would produce four 2-pentenyl furans, i.e., the pentyl furan with a double bond in the n-pentyl group. They should be the *cis* and *trans* 2-(1-pentenyl) furan and the *cis* and *trans* 2-(2-pentenyl)furan (Fig. 2). Dr. Ho and a graduate student synthesized the four compounds in our laboratory and proved their authenticity with infrared, mass and NMR spectrometry.

Sensory evaluations of the two authentic 2-(1-pentenyl) furans showed that when they were dissolved in oil, separately, they could impart a beany and grassy flavor and odor reminiscent of the reversion flavor of soybean oil. The *trans* isomer had a stronger odor and flavor than the *cis* isomer.

Sensory evaluation of the odor and flavor of oil solutions of the authentic samples of *cis* and *trans* 2-(2-pentenyl) furan indicated that they also had a beany and grassy odor and flavor reminiscent of that of the reversion flavor of soybean oil.

Therefore, if linolenic acid in soybean oil undergoes autoxidation to produce either one or all of the four pentenyl furans, they could contribute to the reversion flavor of soybean oil. Since linolenic acid has one more double bond than linoleic acid and, therefore, autoxidizes faster than linoleic acid, it would then be responsible for the development of reversion flavor in soybean oil through the formation of 2-pentenyl furans. We, therefore, proceed to determine whether such pentenyl furans are present in a typical reverted soybean oil. This can be done easily in our laboratory because we already have the four authentic 2-pentenyl furans, and we therefore have their retention times and infrared and mass spectra as references.

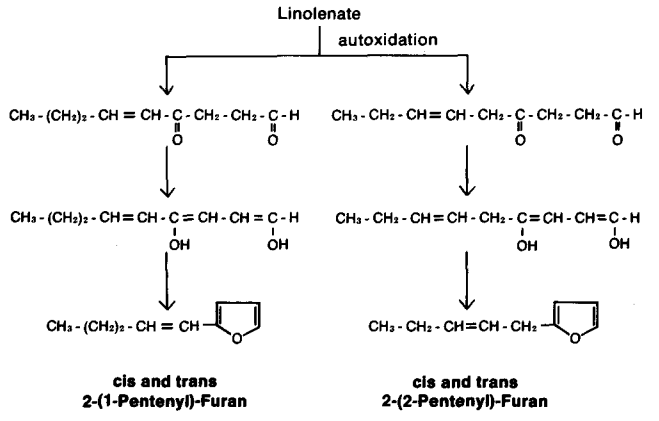


FIG. 2. Mechanisms of the formation of 2-(1-pentenyl)-furan and 2-(2-pentenyl)-furan.

We made the gas chromatogram of volatile flavor compounds isolated from a typical reverted, but not yet rancid, soybean oil. By using the four authentic 2-pentenyl furans, we located where the 2-pentenyl furans would be if they were present in the reverted soybean oil. These fractions were then collected and their chemical structures confirmed with their infrared and mass spectra.

We have definitely proven that *cis* and *trans* 2-(1-pentenyl) furans are present in the typical, reverted soybean oil. However, we have not found any 2-(2-pentenyl) furan, up to this time. Nevertheless, we have proven that linolenic acid can autoxidize to form *cis* and *trans* 2-(1-pentenyl) furans which could then be responsible for the characteristic beany and grassy flavors of soybean oil.

From 1957 until now, in a span of more than 20 years, we constantly and steadily improved and developed our methodology for identification of volatile compounds which are responsible for the flavors of different fats and oils, and high lipid foods. We have applied the method to soybean oil, beef tallow, cocoa butter, roast beef, bacon, roasted peanuts, french fries, baked potatoes and potato chips. Since such studies are important not only to the basic biochemistry of food, but also have practical applications, our research is well supported by NSF, NIH, USDA, trade associations, and private industry. We are therefore fortunate enough to be able to educate a number of graduate students in this area. Many of them now hold key positions in food industry and flavor companies. We are also fortunate to be able to keep our instruments and equipment up to date.

The methodology for identification of volatile flavor compounds that are resonsible for the flavor of foods, consists of four steps: isolation of the volatile flavor compounds from the food; fractionation of the isolated compounds by gas chromatography; the identification of the gas chromatographic fractions by IR, Mass and NMR spectrometry; and finally, organic synthesis of the identified compounds.

Methodology of Flavor Chemistry

With this methodology, we have identified hundreds of compounds in different foods, e.g., 211 compounds in frying fat, 221 in cocoa butter, and 244 compounds in baked potato. The question is, of course, with this large number of compounds, how can a flavor be reconstituted with the compounds identified. This is especially true when the relative concentration of different compounds can affect the final flavor.

Fortunately, most foods have some key compounds primarily responsible for the flavor. It is usually possible to reconstitute the flavor in question, if such key compounds can be identified and used.

This concept can be illustrated by thinking of New York City, which has a large number of buildings, streets, cars, and people. However, these things are not unique to New York City; they are common to other big cities. The key structures which uniquely and characteristically represent New York City are the Statue of Liberty or the twin towers of the World Trade Center. These structures would imme-

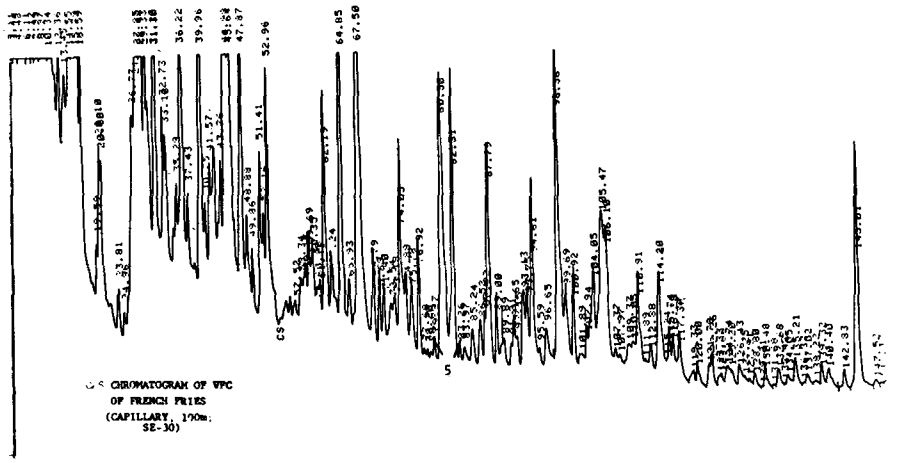


FIG. 3. Gas chromatogram of volatile compounds in MacDonald type french fries.

diately remind you of New York City. In this manner, they are similar to the key compounds in a flavor.

Undoubtedly the flavor of a food which we taste is the total contribution of all the flavor compounds present. However, by the use of the unique and characteristic key compounds of that flavor, we often can produce a flavor which may not be exactly identical to that of the food, but may come very close to it.

Based upon this concept of key compounds, we have developed two different approaches to apply our flavor chemistry methodology to practical applications.

The first approach is to identify only those fractions which have an odor reminiscent of the food in question. For example, it is generally acknowledged that the french fries from MacDonald's quick food stores have a unique flavor. Our objective is therefore to find out what compounds give MacDonald's french fries their unique flavor.

We prepared large amounts of french fries in the laboratory, using the same raw materials as those used by MacDonald's. The gas chromatogram of the volatile flavor compounds isolated from these french fries is shown in Figure 3. The odor of each of the peaks, as it is eluted from the gas chromatograph, was sniffed by an expert panel. The odor description of each of the peaks in the gas chromatogram and the computer printout of retention times were made. The peak with a retention time of 45.64 minutes, with a total area of 0.89% was found to have a MacDonald-like aroma. Also, the peak with the retention time of 80.50 minutes with a total peak area of 0.09% has a typical aroma of the MacDonald's french fries. Therefore, the research is simplified by zeroing into only these two peaks.

The second approach can be illustrated by our study of the flavor of baked potato. We first fulfilled our obligation as academicians by identifying all the flavor compounds in baked potato. We isolated the volatile flavor compounds from some 500 lbs. of baked Idaho potatoes and fractionated the total volatiles into 15 broad fractions. The odor of each fraction was determined. Each fraction was then gas chromatographed again with a different stationary phase into a number of subfractions. The odor of each subfraction was determined, and each of them was gas chromatographed for the third time with another stationary phase into sub-subfractions. The odor of each subfraction was determined and the compounds identified. The peak area for each compound identified was then calculated to give a quantitative guidance for the reconstitution of the baked potato flavor. In this manner, a total of 221 compounds was identified.

By carefully examining the odor description of each fraction, the more important key fractions were selected as shown in Table II. Attempts can then be made to reconstitute the baked potato flavor with the compounds identified in these fractions.

With the methodology I described, we have a fair degree of confidence in handling the volatile flavor compounds of foods. However, the flavor of a food can also be contributed by nonvolatile compounds. Fortunately, a great deal of advancement has been made in the last few years in high performance liquid chromatography. This instrument appears to be ideal for the fractionation of nonvolatile flavor compounds. I would like to use our study of the objectionable bitter and astringent falvor in soybean protein products to illustrate this part of our methodology of flavor chemistry.

A sample as shown in Figure 4 of defatted soy flour was extracted with 60% aqueous ethanol. The extract was concentrated by removing the alcohol and then extracted with ethyl ether to remove the lipid materials. The aqueous extract as shown in Figure 5 was then freeze-dried to get a solid material, which was extracted with methanol. The methanol was then concentrated. Any materials precipitated from the extract after standing at -20 C for 24 hr were then removed. The final concentrate was fractionated by high performance liquid chromatography, using a preparative column.

The HPL chromatogram and the flavor of each of the fractions are shown in Figure 6. The fractions, P3 and P4, had a bitter and astringent taste. Since they were not

TABLE II

Important Fractions of Baked Potato Flavor

Fraction number	Odor Description
5′	Earthy, nutty
5-8(A)	Good earthy, green
5-16	Good earthy, nutty
6-2	Butter, baked potato
6-3	Baked potato, earthy
7-7	Earthy, baked
7-8	Earthy, baked potato
7-9	Earthy, baked potato
7-11	Earthy, good baked potato
8-10	Earthy, baked potato skin, buttery
8-12	Sweet earthy
8-13	Earthy, baked beans
9-18	Good baked potato skin
9-14	Good baked potato skin
10-22	Earthy, baked potato skin

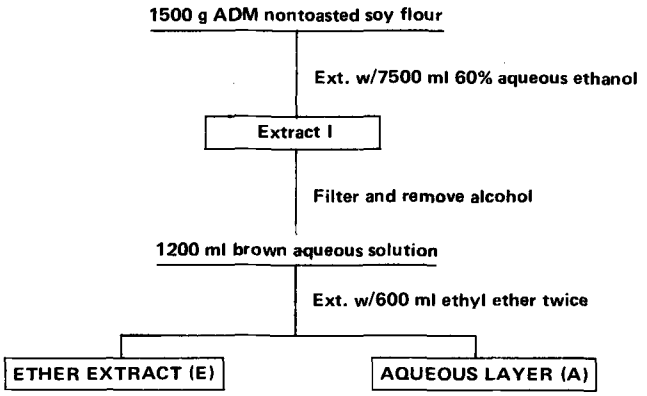


FIG. 4. Extraction of bitter and astringent flavor from defatted soy flour.

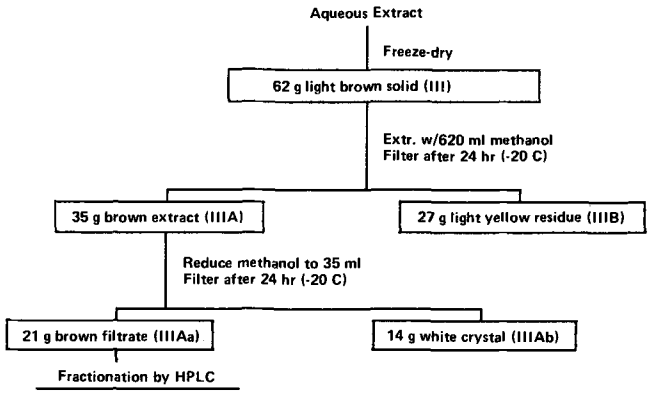


FIG. 5. Concentration of bitter and astringent components.

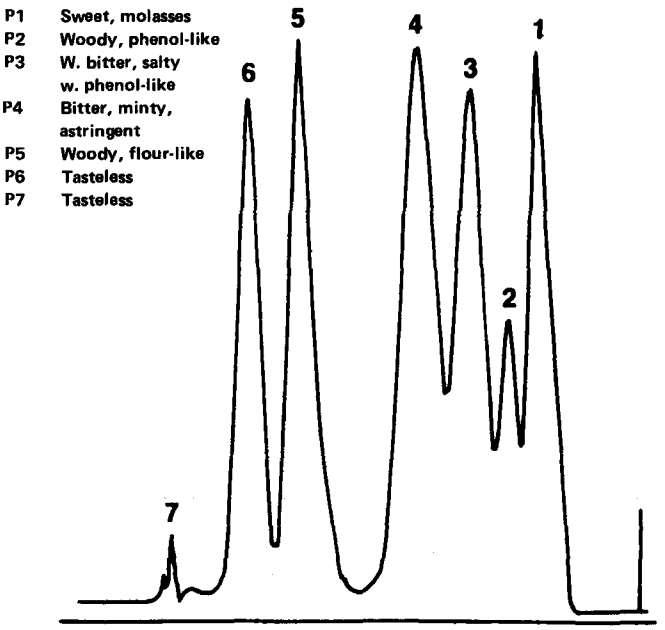
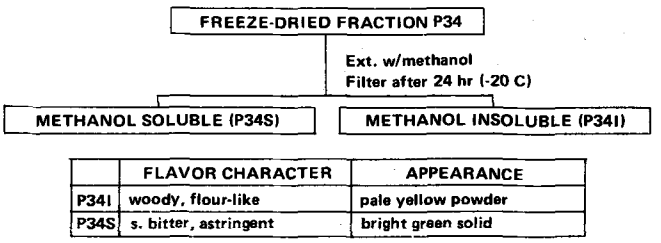


FIG. 6. Preparative HPLC separation of the bitter and astringent flavor.

clearly separated by the preparative column, they were recombined. The recombined fractions, P3 and P4, were freeze-dried to produce a solid material. It was then extracted with methanol and filtered after the solution was allowed to stand at -20 C for 24 hr. The methanol soluble fraction (P34S) had a strong, bitter, and astringent taste as shown in Figure 7.



	FLAVOR CHARACTER	APPEARANCE
P34I	woody, flour-like	pale yellow powder
P34S	s. bitter, astringent	bright green solid

FIG. 7. Reextraction of the bitter and astringent fractions.

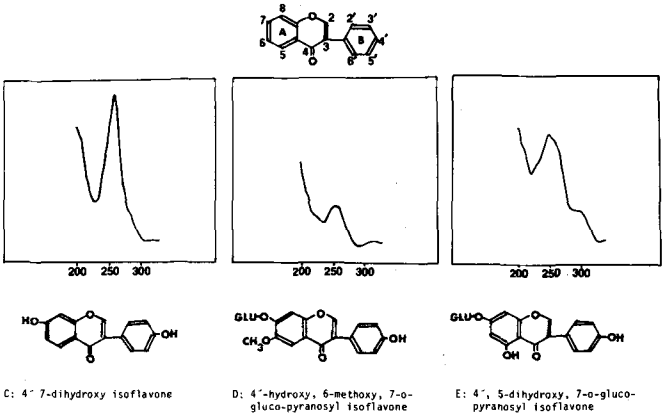


FIG. 8. Identification of the three HPLC peaks as isoflavones.

This fraction was further fractionated by reverse phase high performance liquid chromatography. Three distinctly separate peaks were obtained. The chromatography was repeated until sufficient amounts of each of the three peak materials were collected. They were all white crystalline materials as shown in Figure 8. The three high performance liquid chromatographic fractions were identified as 4′,7-dihydroxy isoflavone, 4′-hydroxy,6-methoxy, 7-0-gluco-pyranosyl isoflavone, and 4′,5-dihydroxy, 7-0-gluco-pyranosyl isoflavone, respectively.

The methodology for the isolation, fractionation and identification of volatile flavor compounds has developed to a stage with considerable satisfaction. Actually, our method has improved to a point that it can now be applied even quantitatively. This methodology enables us to gain basic knowledge in the chemical composition of the flavor of foods. It has also been applied to reconstitute the flavor of foods, to compare the flavor of different kinds of foods, and to understand the change of flavor during processing or storage. The methodology can also be used to study objectionable flavors of foods in order to retard or eliminate their formation.

The methodology for the study of the nonvolatile flavor compounds in food is still in its infancy. We hope we will be able to develop a methodology for the nonvolatile flavor compounds in the next decade which will be as satisfactory as the methodology we have for the volatile flavor compounds.

ACKNOWLEDGMENT

I would like to acknowledge that the research reported in this paper is the total effort of a large number of graduate students, postdoctoral fellows, and assistant professors who were my associates during the last 19 years at Rutgers University. I can count 45 persons who contributed to this program. It was the extremely intelligent and dilligent work of these colleagues that made this paper possible.