A Gas Chromatographic Method for Measuring Rancidity in Vegetable Oils

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Abstract

A simple gas chromatographic procedure was developed for measuring the degree of rancidity in cottonseed oil and other vegetable oils. The procedure employs an internal standard for quantitating the amount of *n*-pentane in the oil samples and relates this quantity to organoleptic panel tests. The precision is good, and the results correlate well with panel tests. The procedure is very sensitive to detection and quantitation of oxidative changes in oils. Its use minimizes manipulation of the sample and thus avoids alteration of the rancidification products or the state of oxidation.

Introduction

THE TESTS MOST COMMONLY used for determining rancidity in vegetable oils and animal fats are the Kreis (16,17), the 2-thiobarbituric acid (1,22), and the peroxide value (24,25) tests. Photochemical methods, total carbonyl determinations, and aldehyde tests are among the other methods used.

The above tests are not always precise, and sometimes fail to correlate with organoleptic measurements of rancidity. Extensive studies (7,18,19) have shown that fats are described as rancid at various peroxide levels. However, a case was reported where peroxide values and rancid flavors and odors were not closely associated (20). Consequently, we decided to develop, an analytical method that would be more precise than other tests and would correlate well with the results obtained by flavor and odor testing panels. Gas chromatography was selected as the most promising approach. Also, autoxidation products related to rancid flavors and odors have been characterized by gas chromatography (3–5,12,14). A definition of flavor profile by gas chromatography has been suggested by Van der Kloot and co-workers (26). Nawar and Fagerson (21) have compared the direct sampling method with gas chromatography as an objective method for flavor measurement.

Experimental

Reagents

The reagents were pure-grade (99.9% purity) *n*-pentane, from the Phillips Petroleum Company, and reagent-grade *n*-Octanol, from the Eastman Chemical Company.

Apparatus

The analytical balance was used in all weighings. The 1-ml volumetric test-tube shaped flasks were stoppered with ground glass stoppers. The 10-μl hypodermic syringe was fitted with a device that enabled sample size to be reproduced accurately. The gas chromatograph was an F & M Scientific Company model 609 equipped with a hydrogen-flame detector. The recorder was a Sargent SR operated at 1 mv and 0.5 in./min chart speed.

Preparation of Standards

Pure-grade *n*-pentane was added to fresh cottonseed oil to obtain a standard oil containing approximately 1000 ppm pentane. Quantitative dilutions of this standard oil were made with fresh oil to obtain a series of standards with a concentration range of 0 to 1000 ppm added pentane.

Sampling

The primary test material was refined, bleached and fresh deodorized cottonseed oil, obtained in 1-gal cans on special order from Corn Products Company. No antioxidants were added to the oil. Four cans were obtained at one time. One can was opened each week, and the contents were transferred to four 1-qt bottles left open to the atmosphere and stored at 77F in a constant-temperature room. Every 4 weeks another set of cans was received. Each batch received was analyzed immediately to determine the initial pentane concentration, which was usually between 10 and 20 ppm. Periodically bottles were selected for analysis and removed from the storage room. One portion was removed from the bottle for gas chromatographic analysis and another portion placed in small, thoroughly cleaned and baked, ground glass covered sample bottles, for evaluation by the organoleptic panel.

Organoleptic Evaluation

Since fatigue is a major problem in tasting oily foods, we selected odor evaluation rather than flavor. An organoleptic test panel, consisting of six selected individuals, was set up to determine the state of autoxidation in the oils by means of odor evaluation. During the period of study, 8 months, the makeup of the panel changed four times. The members of the panel were instructed to give their findings a numerical rating according to the following scale: *0*, not rancid; *1*, very slightly rancid; *2*, slightly rancid; *3*, moderately rancid; *4*, strongly rancid; *5*, very strongly rancid. The numerical rating assigned to each sample by the panelists was averaged to determine the average rating for each sample.

Gas Chromatographic Determination of Pentane

The gas chromatograph was used with the following operating parameters: column, 10 ft, Carbowax 20M, 15% on Chromosorb P; column temperature, 150C; injection port temperature, 250C; detector block temperature, 150C; carrier gas, helium, flow rate 75 ml/min; detector hydrogen flow, 35 ml/min; detector airflow, 275 ml/min; range 10; attenuation, variable, depending on quantity of pentane present.

Sample Preparation

Approximately 1 ml of oil was weighed into glass-stoppered 1-ml volumetric flasks and the weight recorded. As an internal standard, *n*-octanol was added to the sample. The quantity added is immaterial but must be reproducible from sample to sample. The 10-μl syringe fitted with the special adaptor delivered approximately 0.8 μl of octanol to each sample. The samples were then mixed thoroughly by shaking.

Analysis

Approximately 0.6 μl of prepared sample was injected into the gas chromatograph. Under the operating conditions used, the retention time was approx-

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imately 1 min for pentane and 18 min for octanol. In the more rancid samples the oxidation products required up to 60 min to be eluted.

Calculations

The peak height ratios between the pentane and the octanol were used to determine the pentane concentrations. The peak height of the pentane was divided by the peak height of the octanol. This ratio was then divided by the weight of oil taken (peak height ratio per gram of oil), to provide a corrected ratio. The ratio of peak areas instead of heights can also be used.

A plot of peak height ratio per gram of oil versus ppm of added pentane on linear graph paper provided a linear calibration curve, from which the pentane content for the unknowns was obtained. Figure 1 shows the calibration curves obtained from the standards.

Results and Discussion

It is well known that the human response to rancidity is a complex process and that the rancid flavors and odors are in themselves very complex mixtures of chemical compounds. No one component in an odor is responsible for its subjective effect. The effect is due to a combination of all the components present. The difficulties that arise in trying to define a flavor have been described by Nawar and Fagerson (21).

The ultimate in an analytical procedure to replace human evaluation and thus to provide a complete and purely objective evaluation of rancidity would involve an exhaustive analysis of the rancidity products of the oil. This would be a monumental task.

The analytical methods available today for defining rancidity are, of necessity, designed to analyze for one product only or for one type of product. For example, in the thiobarbituric acid test the rancid oil is tested for a single oxidation product, which has been shown to be malonaldehyde (22,23). Although the gas chromatographic test follows the same precarious approach to flavor definition, namely, the determination of only one product, *n*-pentane, which in itself contributes little flavor or odor, gas chromatography opens the way to a more extensive analysis of oxidation products and hence a more precise, reliable, and definitive expression for rancidity.

An advantage obtained by directing analyzing a sample of oil by gas chromatography is the elimination of preliminary sample manipulation, which might alter the composition of the oil before analysis. The addition of heat, as in steam distillations, or the addition of solvents, as in extractions, introduces the possibility of altering the composition of the original sample. The gas chromatographic method allows the analyst to analyze the sample with only the addition of the octanol internal standard as preliminary treatment. The oil is injected directly into the chromatograph, and the lower-molecular-weight oxidation products are volatilized in an oxygen-free (helium) atmosphere.

The possibility of thermal degradation of the oil and/or some of the oxidation products in the injection port is minimal or nonexistent, as can be seen by comparing the chromatograms in Figure 2. Figure 2A shows the chromatogram for rancid cottonseed oil. Acetaldehyde, propionaldehyde, butylaldehyde, *n*-pentanal, *n*-hexanal, and *n*-decanal were identified in addition to pentane.

Pentane was chosen as the basis for analysis because of its observed more rapid increase in concentration as autoxidation progressed. Figure 2B shows that the chromatogram for fresh cottonseed oil was "clean" except for the presence of a small pentane peak.

Pentane was also detected in fresh peanut oil, corn oil, and soybean oil. Since its concentration increased rapidly during autoxidation, it served well as an analysis peak for the early as well as the later stages of autoxidation. Measurable changes in the concentration were observed within the first week or two after the oil was exposed to air at 77F and well before taste panel members detected the rancid odor.

The presence of *n*-pentane and other hydrocarbons has been postulated and reported by many workers. Frankel and co-workers (10) and Evans (8) predicted the production of hydrocarbons from the autoxidation of fatty acids. Evans et al. (9) detected the presence of short-chain hydrocarbons from the autoxidation of soybean oil. Horvat and co-workers (13) determined that 90% of the hydrocarbons produced by the autoxidation of methyl linoleate was pentane.

Buttery et al. (2) found C_1–C_5 saturated hydrocarbons in dehydrated potatoes from the autoxidation of linoleic acid, which is the principal fatty acid in potato lipids. They suggested that *n*-hexanal was the source of these hydrocarbons, their production being catalyzed by fat peroxides.

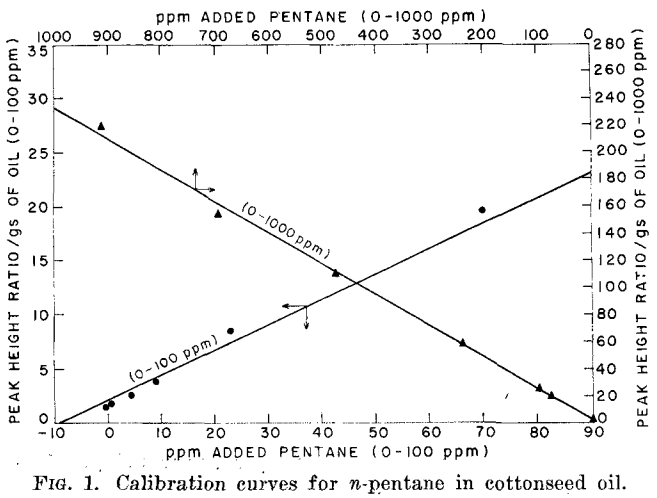


FIG. 1. Calibration curves for *n*-pentane in cottonseed oil.

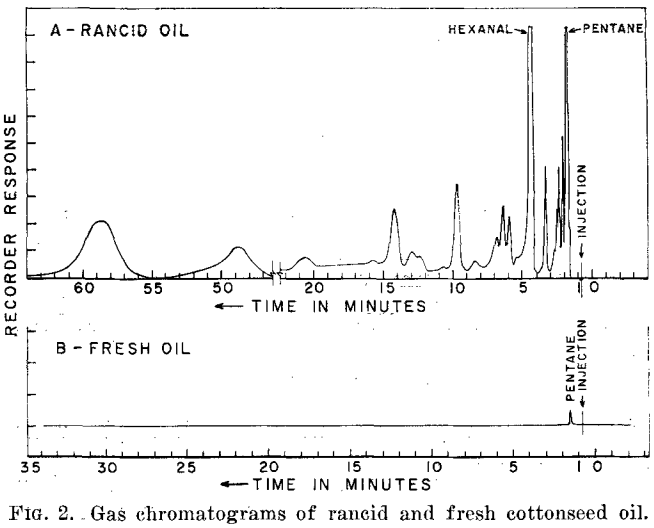
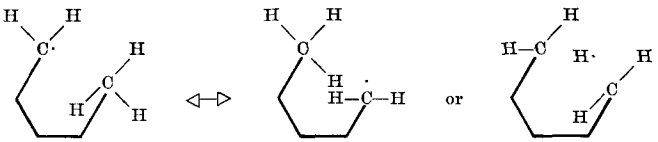


FIG. 2. Gas chromatograms of rancid and fresh cottonseed oil.

In trying to purify *n*-hexanal by distillation and preparative gas chromatography, we found that pentane was always present in the purified hexanal. The pentane concentration increased somewhat on standing, and carbon monoxide was readily detected and identified in the headspace gas above the hexanal. The mechanism for the production is probably like that described by Kerr and Trotman-Dickenson (15) for the photoinitiated formation of hydrocarbons from aldehydes.

The decarbonylation of *n*-hexanal is probable. The free radical $CH_3(CH_2)_4\cdot$ can be depicted to give rise to two equivalent free radical structures or a hybrid structure that lends stability to the radical. Such structures would be:



The production of *n*-pentane by the above mechanism suggests the formation of *n*-decane. That none was detected in our analyses could be explained on the basis that the radical concentration was too low for two pentane radicals to be close enough to unite.

Octanol was used as the internal standard because it was eluted at a position where no other component was eluted. When oils with other than corn, cottonseed, or soybean origin are tested, it is possible that a product of autoxidation may interfere with the octanol peak. In these cases some other suitable noninterfering compound could be used as the internal standard.

In the internal standard method of analysis, a known amount of internal standard is added to a known amount of test sample. If the same amount of both materials is used in all samples, the peak height of octanol for a given sample size will be constant. To simplify sample preparation, the weight of oil taken was not fixed but the differences in weight were corrected for by dividing the peak height ratio by the grams of oil used. Also, since weighing a small amount, such as 1 μl, is rather difficult, the octanol was added to the oil with a 10-μl syringe fitted with a device to provide reproducible filling of the syringe. Even though the exact amount of octanol is not known, the same amount was added to all samples. The careful reproducibility of this small amount of octanol is essential for obtaining precise results. The precision of the gas chromatographic method was 8%.

It was found to be advantageous to use a plug of glass wool in the injection port positioned such that the tip of the hypodermic needle was within the plug o ninjection. This technique facilitated rapid volatilization by providing more surface for vaporization, and it helped retain the relatively nonvolatile oil. Baseline stability was unaffected by the retained oil.

The calibration lines in Figure 1 were plotted by using calculated points determined from the equation for the best-fitting straight line derived by the least squares method of calculation. The equation for the line is:

$$y = 2.15 + 0.23x$$

where x = ppm pentane and y = peak height ratio per gram of oil.

The straight lines do not pass through the origin because the fresh oil had a residual amount of pentane, approximately 9 ppm. The plot shows the response was quite linear over the range studied.

For each sample, a plot of the rise in pentane concentration against storage time gave a straight line from the initial pentane concentration, which was usually about 10 to 20 ppm, to the final concentration, 460 ppm. The line may begin to curve after 460 ppm, but insufficient samples were run to verify this.

The limit of chromatographic detectability was estimated to be 25 to 50 ppb of pentane, based on a minimum response twice the background noise level and on a sample size of 1 μl. This corresponds to approximately 10^{-13} mole of pentane per microliter of sample.

TABLE I
Correlation of Organoleptic Panel Tests and Gas Chromatographic Analyses

Pentane, ppm	Panel rating	Description
0–50	0.00–0.45	Not rancid
50–300	0.45–1.33	Very slightly rancid
300–460	1.33–2.33	Slightly rancid

When the organoleptic test results were divided into three groups according to the rating scale used for the organoleptic tests, i.e., *0*, not rancid, *1*, very slightly rancid, and *2*, slightly rancid, definite pentane concentrations fell into each group. There was not a point-to-point correspondence with the pentane concentrations, because the average rancidity ratings varied widely within each group. However, the ratings did not overlap from one group to the next. Table I depicts the three groups and shows the range of values found for each. The wide variation in the average rancidity ratings was a reflection of the wide variation in the individual scoring among the various panelists. Their results are highly subjective and susceptible to many variations, and, in addition, the same panelists were not used throughout the study. The results are plotted in Figure 3.

The data indicate a correlation between pentane concentration as determined chromatographically and rancidity as evaluated by the organoleptic panel. Cottonseed oil with 0 to about 50 ppm pentane had a fresh, or neutral odor. Cottonseed oil with about 50 to 300 ppm pentane had almost neutral odor. Organoleptically detectable odor changes began with a concentration of 50 to 300 ppm pentane. Although odor changes were noted in samples containing less than 300 ppm pentane, they were not generally described as rancid.

Over the period of time the test was carried out, 8 months, the highest concentration of pentane found in cottonseed oil samples was 460 ppm and the highest rating assigned was 2.33, slightly rancid.

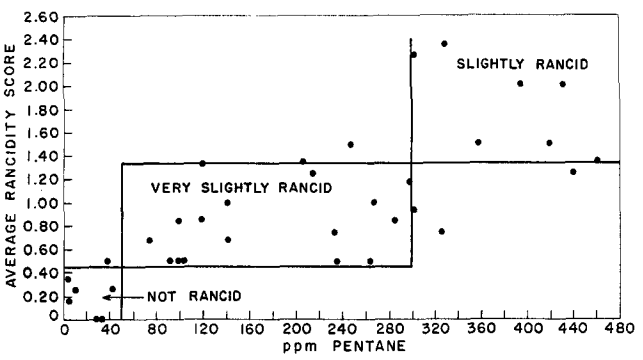


FIG. 3. Correlation of organoleptic panel tests and gas chromatographic analyses for pentane.

Although the method is generally applicable to other types of vegetable oils, the specific correlations found in this series of tests with cottonseed oil are not. Correlations must be established independently for each type of vegetable oil. Preliminary test of soybean oil indicated a threshold value of around 68 ppm pentane and a strong, detectable odor at 150 ppm pentane.

The straight-line graphs obtained from plotting the pentane concentrations against storage time indicate that the expected shelf life of cottonseed oil can be predicted with a minimum number of storage tests during the early stages of autoxidation.

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REFERENCES

1. Bernheim, F., M. L. C. Bernheim and K. M. Wilbur, J. Biol. Chem. *174*, 257 (1948).
2. Buttery, R. G., C. E. Hendel and M. M. Boggs, J. Agr. Food Chem. *9*, 245 (1961).
3. Chang, S. S., JAOCS *38*, 669 (1961).
4. Ibid. *38*, 671 (1961).
5. Craig, B. M., and N. L. Murty, JAOCS *36*, 549 (1959).
6. Crossley, A., T. D. Heyes and G. J. F. Hudson, JAOCS *39*, 9 (1962).
7. Dugan, L. R., Am. Perfumer Aromat. *73*, 47 (1959).
8. Evans, C. D., Proceedings of the Flavor Chemistry Symposium, Campbell Soup Co., 1961.
9. Evans, C. D., P. M. Cooney and H. J. Dutton, presented at the AOCS Meeting Chicago, October, 1961, paper No. 37.
10. Frankel, E. N., J. Nowakowski and C. C. Evans, JAOCS *38*, 161 (1961).
11. Herb, S. F., P. Magidman and R. W. Riemenschneider, JAOCS *37*, 127 (1960).
12. Hoffmann, G., JAOCS *38*, 1 (1961).
13. Horvat, R. J., W. G. Lane, H. Ng and A. D. Shepard, Nature *203*, 523 (1964).
14. Horvat, R. J., W. H. McFadden, H. Ng, D. R. Black, W. G. Lane and R. M. Teeter, JAOCS *42*, 1112 (1965).
15. Kerr, J. A., and A. F. Trotman-Dickenson, J. Chem. Soc. 1960, p 1602.
16. Kreis, H., Chem. Z. *23*, 802 (1899).
17. Ibid., *26*, 897 (1902).
18. Lea, C. H., Chem. Ind. London, No. 49, 1953, p 1303.
19. Lips, H. J., Food Can. *12*, 9 (1952).
20. Naumann, H. D., D. E. Brady, A. Z. Palmer and L. N. Tucker, Food Technol. *5*, 496 (1951).
21. Nawar, W. W., and I. S. Fagerson, Food Technol. *16*, No. 11, 107 (1962).
22. Patton, S., and G. W. Kurtz, J. Dairy Sci. *34*, 669 (1951).
23. Sinnhuber, R. O., T. C. Yu and T. C. Yu, Food Technol. *11*, 104 (1957).
24. Standsby, M. E., Ind. Eng. Chem. (Anal. Ed.) *13*, 627 (1941).
25. Wheeler, D. H., Oil Soap *9*, 89 (1932).
26. Van der Kloot, A. P., R. I. Tenney and V. Bavisotto, Proc. Ann. Meeting Am. Soc. Brewing Chemists, 1959, p 96.

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