Determination of Pentane Formed during Autoxidation of Oils Contained in Solid Samples

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ABSTRACT AND SUMMARY

A convenient and practical technique for the measurement of pentane formed during peroxidation of solid materials containing unsaturated oils was developed. The test has a sensitivity below .1 parts per million (ppm) and can be used to monitor shelf-life of a variety of products. A special glassware was designed to perform a single extraction-concentration step. The pentane is recovered in 2 ml of hexane and analyzed by gas chromatography. This method correlates well with taste panel scores.

INTRODUCTION

Several tests have been developed that measure the rancidity of fats and oils. Some are based on the characteristic reaction of certain aldehydes like the Kreis test (1,2) and 2-thiobarbituric acid (TBA) test (3,4), while others are based on the measurement of total carbonyl compounds (5-7), or ketones (8). Still others, like the peroxide value (9,10), measure the total reactive capacity of the products of oxidation. Although these tests have been modified, the underlying principles have changed little over the years.

Of the above procedures, peroxide value and TBA test are probably the most widely used. The others are used mainly in specific applications of flavor research. One different possibility was explored by Scholz and Ptak (11) in a method based on the chromatographic measurement of pentane formed as a breakdown product of the autoxidation of linoleic acid. This study, done on cottonseed oil, used the direct injection of the oil into the chromatographic column. The method was modified by Evans and collaborators (12). In both reports, the authors showed good agreement with flavor scores.

In our laboratory, the need developed for a reliable rancidity test to be used in shelf life studies of a variety of products. In all cases, the oil was contained in a solid matrix such as peanuts, almonds, and other nut-containing products. In most cases, substantial amounts of other ingredients such as sugar were also present. The pentane measurement presented the advantage of being a terminal product of oxidation and by its nature, less subject to further interaction with other food ingredients. Nevertheless, this method did have some difficulties: the sample preparation required some extracting or pressing of the oil. The ingredients in the commercial products are not uniformly distributed; consequently, larger samples are required for meaningful analyses. Also, the gas liquid chromatography (GLC) columns were easily contaminated by repetitive injections producing changes in retention times and questionable quantitative results. This was of particular concern for shelf life studies when samples were compared at monthly intervals. A relatively simple system was developed for a one step extraction-concentration procedure applicable for shelf life studies of the above-mentioned materials. The general principle involved is to distill the sample in water with addition of a known volume of solvent similar to pentane (hexane). The solvent carries the pentane and separates from the water by differences due to density. This clean extract is used for the analysis. A preliminary report of this method was presented (13).

During recent years, a similar principle had been studied successfully (14,15), analyzing by GLC the volatile components producing during peroxidation. However, after working for more than 5 yr with our test, we feel that it has applications with desirable advantages in the area of shelf life studies of nut-containing snacks and other processed foods.

EXPERIMENTAL PROCEDURES

Sample Preparation

For foods that disperse easily by melting or dissolving in boiling water, the sample is cut into pieces small enough to enter the T24/40 neck of a flask. For whole nuts or products not dispersible in hot water, it is necessary to grind the material small enough to pass it through an ASTM No. 8 mesh sieve prior to weighing directly in a flask. For snack items, it is preferable to use no less than one whole unit per analysis and no less than three complete analyses per sample.

Special Glassware

A special receiver-condenser was designed for this procedure. It allows for a continuous distilling and condensing of the sample, partitioning between the solvent and water inside of the condenser (Fig. 1). Also required is a 500 ml round bottom flask and a 30 cm Graham condenser. All the glassware had T24/40 joints.

Extraction Procedure

A sample containing between 5 and 15 g of fat is weighed directly in a 500 ml round bottom flask; a magnetic bar, 250 ml of water, 3 ml of acetic acid, and 2 ml of hexane are then added. The flask is attached to the receiver and a Graham condenser is placed on the top of the receiver. Distilled water (15-20 ml) is added through the condenser to fill the receiver elbow.

The flask is heated with a heating mantle controlled with a rheostat that has been previously calibrated to start boiling water above the condenser in 20 min. The liquid is agitated by a magnetic stirrer placed under the mantle. The boiling continues for 20 min, after which the mantle is removed and the flask is allowed to cool for another 20 min.

During the operation, the condensers are cooled by circulation of water or by a mixture of water and propyleneglycol at 4 C. When the flask is cooled, the Graham condenser is removed and the hydrocarbon that has distilled is collected with a capillary pipette and transferred to a centrifuge tube that contains 2 ml of water; the tube is shaken and centrifuged, and the water discarded. A few crystals of Na_2SO_4 anhydrous are added to the test tube, letting it stand for 10 min, after which the sample is ready for injection in the gas chromatograph.

Gas Chromatography

The instrument used was a Hewlett-Packard model number 7620 provided with electronic integrator and automatic injector. Each sample was prepared in triplicate and each vial was injected three times, a total of nine injections per sample. (This large number of injections can be easily handled because the method is applicable to

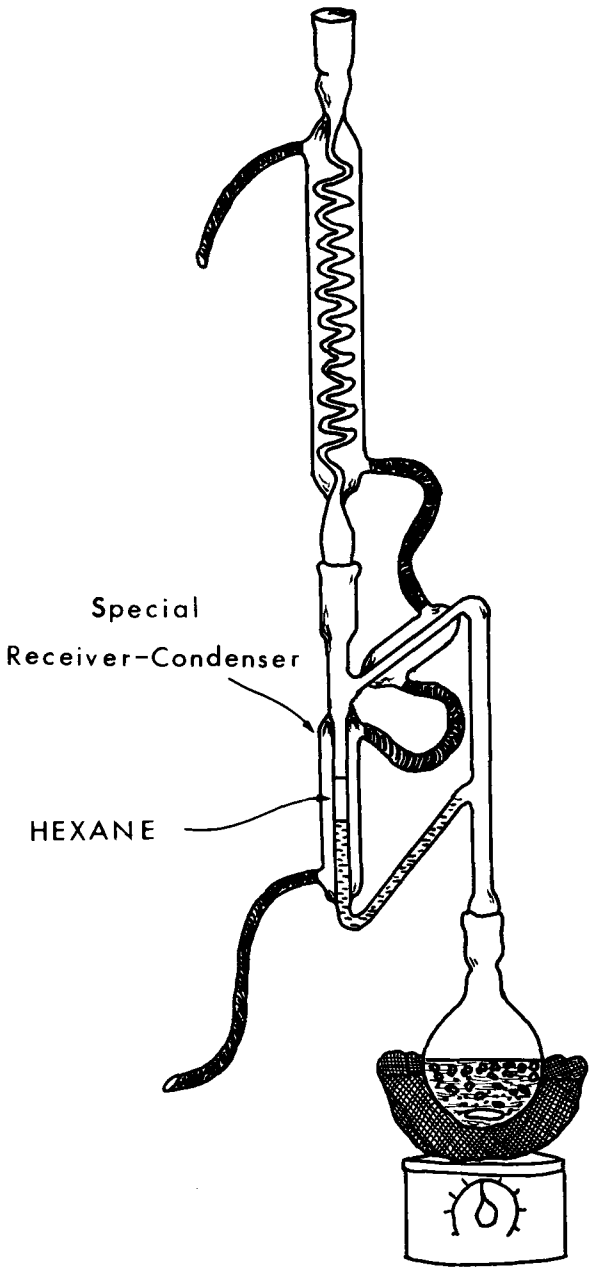

FIG. 1. Apparatus used for the isolation of pentane, including the special receiver condenser.

instruments with automatic injector devices. Precision and accuracy are presented later.) The gas chromatograph was operated under the following conditions: column: 8 ft x 1/8 in. stainless steel 15% SE-30 on Chromosorb P-AW 100/120 mesh; column temperature 80 C isothermal for the first 4 min after which the integration stops and starts programming at 20 C/min up to 230 C (this programming is only for the cleaning of the column); injector temperature at 220 C; flame detector block at 280 C; carrier gas nitrogen at a rate of 40 ml/min; detector with air flow of 300 ml/min and a H_2 flow ca. 30 ml/min, slightly adjusted daily, to produce a constant number of counts in the integrator per the same volume of standard; range of the electrometer 10^3.

Injection and Calculation

Two microliters of the extract were injected in all cases. An external standard technique was found satisfactory and preferred over the internal standard technique. The external standard was a solution of pentane in the chosen solvent in a proportion 1 to 10,000 v/v which is equal to .06262 mg of pentane per ml of solution (or .6262 to 10,000 w/v). The values in parts per million were calculated as follows: ppm = [(1000 x C x V x H)/(S x A x M)] x F; in which: A = Average counts for three or more injections of STANDARD; C = Average counts for the sample; M = Size of the injection in microliters; S = Weight of the sample in grams; V = Volume of the hexane or heptane that was placed in the flask expressed in milliliters; H = Micrograms of external standard injected in the volume M; and F = Recovery correction factor calculated for that solvent and that type of sample.

The Samples

Several fats, peanuts, almonds, and products made with these materials were tested; this report contains our results on peanuts and almond products. The samples consisted of: (A) experimental samples containing (30-40%) roasted ground peanuts, sugar, cocoa liquor, cocoa butter, and flavoring agents (in this product, most of the substrate for oxidation was peanut oil); (B) Spanish No. 1 peanuts (a commercially available type), subjected to a dry roast; (C) a sample equal to (A), made under different conditions and population of peanuts; (D) another experimental sample with similar composition with larger proportion of peanuts (45-50%); and (E) a confectionery item containing 10-15% of chopped almonds enrobed in chocolate. The weight of the sample for each analysis was 15 g for roasted peanuts and 45 g for the experimental products. Samples were stored under "average" environmental conditions to study deterioration. These conditions were: 25 C with 50% RH. Control samples were stored sealed in a deep freezer (-20 C). The deterioration was followed as a function of time.

The Taste Panels

Some experiments were done simultaneously with a taste panel. Fresh or frozen samples were used as references and tested with a multiple comparison difference test that work in combination with a Hedonic scale (16).

TABLE I

Increase in Pentane Concentration and Flavor Score of Five Nut Products upon Storage

A. Peanut product			B. Peanuts			C. Peanut product			D. Peanut product			E. Almond product		
Days	ppm Pentane	Flavor scores	Days	ppm Pentane	Flavor scores	Days	ppm Pentane	Flavor scores	Days	ppm Pentane	Flavor scores	Days	ppm Pentane	Flavor scores
0	3.28	5.08	0	1.04	5.00	0	0.19	5.00	0	0.16	5.00	2	0.15	5.05
21	4.31	5.58	9	2.02	5.75	28	12.07	7.07	28	0.62	5.63	28	1.27	5.53
29	9.85	6.42	16	4.57	6.58	62	25.91	7.32	62	12.50	6.46	56	3.18	6.32
35	17.86	8.08	23	6.98	6.67	110	30.53	7.91	110	27.40	7.41	112	5.02	7.43
			37	12.92	6.83							155	5.56	8.00
r = .995[a]			r = .812			r = .927			r = .968			r = .991		

[a]r = Correlation Coefficient.

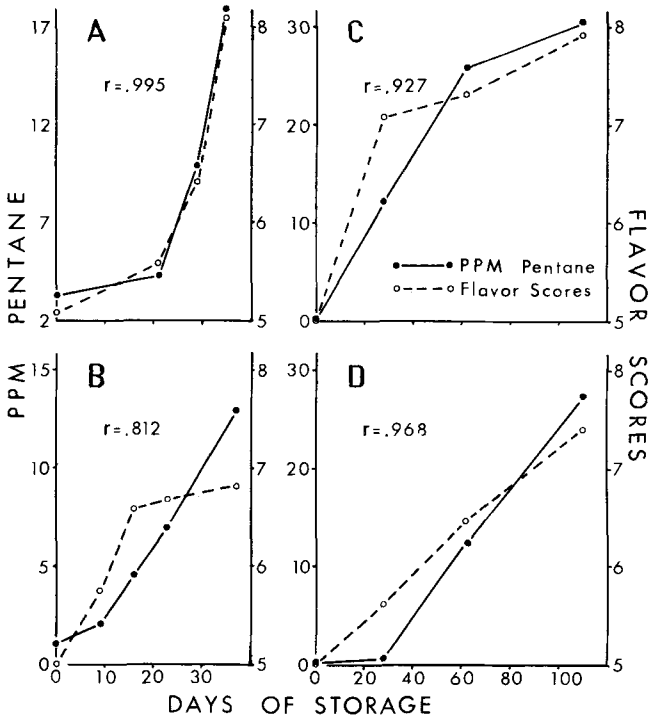

FIG. 2. Comparison of ppm of pentane and flavor score averages of peanuts (B) and peanut products (A,C,D) during storage.

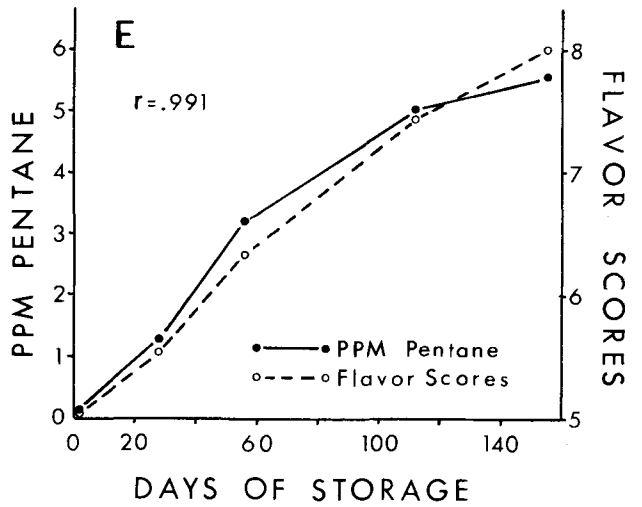

FIG. 3. Comparison of ppm of pentane and flavor score averages of an almond product (E) during storage.

In each test one external and one internal control was used. The Hedonic scores were as follows: Extremely better = 1; Much better = 2; Moderately better = 3; Slightly better = 4; Equal to the control = 5; Slightly inferior = 6; Moderately inferior = 7; Much inferior = 8; Extremely inferior = 9.

A group of twelve experienced panelists was used for all Hedonic testing and were specifically instructed to "look" for rancidity. The samples of roasted peanuts were ground before being given to the panel to avoid the influence of different textures.

Precision and Accuracy

The precision, measured by Coefficient of Variation (CV) and accuracy, measured by percent recovery, were checked separately for the injection system, the distillation, and the interaction with the sample.

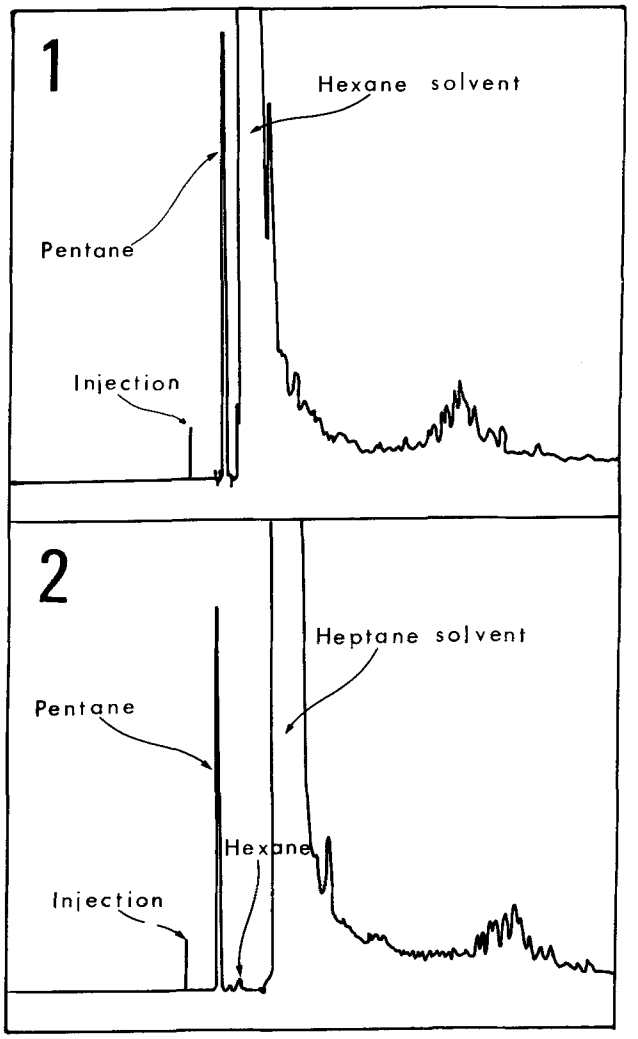

FIG. 4. (1) Typical chromatogram of a rancid peanut product. The solvent is hexane. (2) The same sample analyzed with heptane as solvent. The small peak of hexane is also present.

(a) The linearity of the GLC response and the injection system was checked from 10% to 1000% of the standard and was found to be in good agreement with the expected values. (b) The distillation was studied by placing 2 ml of standard solution in 250 ml of water and performing the complete technique. (c) The effect of the sample on the precision and accuracy was observed, starting with fresh samples and determining the amount of pentane (usually very low) then repeating the same determination with 2 ml of standard solution added to the flask instead of 2 ml of solvent (hexane). Each one of these tests was done twelve times. Percent recovery and recovery factors were calculated.

After the twelve determinations, it was found that: (a) CV = 1.8 for the injection and integration of the system; (b) CV = 7.3 for distillation of standards in water; and, (c) CV = 6.9 for distillation of standards with a sample of peanuts; a percent recovery of 64.4%. Consequently, a correction factor of 1.55 was applied in our work.

With solid samples containing 30-50% peanuts, it was found that pentane increases with rancidity from .1 ppm to 20 ppm or more. This wide range of values makes the variation insignificant. Also, the close values for the CV of the distillation with and without the sample (7.3 and 6.9) indicate that there is no effect in the reproducibility of the test and that it is mainly related to the temperature control of the mantels and condensers.

Initially, heptane was used as a solvent and hexane as an

internal standard. However, measurable amounts of hexane were present in most samples and its rate of formation was found to be different from that of pentane (Fig. 4). This problem and the acceptable results obtained with an external standard system favored the use of hexane as a solvent.

RESULTS AND DISCUSSION

The five independent experiments of the pentane determination and the sensory evaluation flavor scores are presented in Table I and in Figures 2 and 3.

Comparing the two types of information, there appears to be a close correlation as is indicated by the Correlation Coefficients (r) reported in the table.

These experiments were conducted during several years of research work on product stability, and during this period the makeup of the taste panel changed due to the turnover of members. It is possible that the small differences in the intensity of scores between experiments could be related to panel turnover. Also there are some differences in the way the rancid flavor is perceived, and this is related not only to differences among panelists and the type of oil, but also to the solid matrix in which the oil is retained and the speed of the flavor release in the mouth. Some of these factors could have some effect in lowering the Correlation Coefficient on Experiments B and C.

The formation of pentane is dependent on the type of lipid substrate in the food; consequently, the ratio between flavor scores and ppm of pentane is unique for each particular type of sample. However, after this relation is established for a product, the flavor score can be predicted with accuracy.

Also, recovery factors could change slightly with different substrates or operating conditions; however, for these studies it is more important to know the relative values within the study than the absolute amount of pentane.

The inertness of pentane and the complexity of most fabricated products are two factors that substantially increase the practical applications of this method because in most samples there are sufficient interfering materials to make other analyses difficult and unreliable.

With products similar to those presented in this report, we have tested not only experimental samples but also storage conditions in warehouses, packaging materials, changes in formulations, and processing conditions. In many cases, it was not necessary to run a taste panel.

We believe that for most applications with solid foods, this is a very reliable and convenient test.

ACKNOWLEDGMENT

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