Determination of Residual Hexane in Solvent Extracted Meals

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ABSTRACT AND SUMMARY

A simple modification of the volatilization procedure improved the reproducibility and covered a wide range of residual hexane in meal with good precision. A comparison study showed that the modified volatilization method released and assayed more residual hexane from the meals than did the direct gas chromatographic technique.

INTRODUCTION

For determining residual hexane in solvent extracted oilseed meals, Dupuy and Fore (1) described a volatilization procedure in which a meal sample was heated in a sealed bottle, and then a headspace gas sample was injected into a gas chromatograph (GC) for analysis. Later, they (2) described another procedure in which a meal sample was placed in the heated injection port of a GC, and the hexane eluted from the sample directly. This has been called the direct GC method. The first method was easier to operate and more flexible in choosing sample sizes and the operating conditions than the direct GC method.

When reviewing the methods for the purpose of selecting one for use in the laboratory of Food Protein Research and Development Center (FPRDC) at Texas A&M University, the direct GC method seemed to have some disadvantages: it required modification of the GC equipment to handle a modified sample tube, and it increased the wear and tear on the threaded connections of the injection port of GC equipment. However, the reproducibility of the volatilization procedure was questionable. This variation was reported to be as large as ± 20% (1), which is undesirable for an analytical method. It was found that the large apparent deviation of residual hexane in the meal, as determined by the volatilization method, was caused by the unevenly distributed water introduced on the top of the meal sample in the serum bottle before heating. A simple modification was devised and tested in the laboratory of FPRDC.

[1]Deceased.

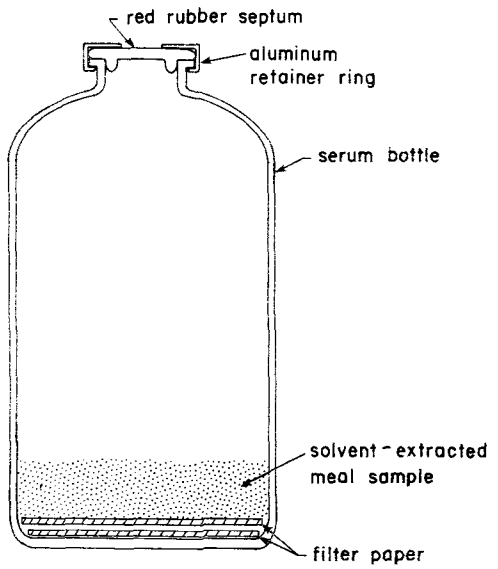

FIG. 1. A cross-sectional view of an assembled serum bottle used in the modified volatilization method.

EXPERIMENTAL PROCEDURES

Materials

Hexane extracted glandless cottonseed meals were prepared on a laboratory scale by the Food Protein Research and Development Center of Texas A&M University. These meals have been desolventized at various temperatures and vacuum conditions.

Modified Volatilization Procedure

The main idea of the proposed modification of the volatilization method is to have the water spread evenly by using the filter paper as shown in Figure 1. Two layers of Whatman #40 filter paper cut to the ID of the serum bottle were placed into the serum bottle which has a volume of 160 ml. The filter papers were wetted with 0.5 ml of water, then a 2 g (± 0.01 g) meal sample was introduced into the serum bottle. The serum bottle was immediately sealed and placed in a thermally equilibrated sand bath for 2 hr. The sand bath had been preheated and kept in a 125 C oven. This sand bath can hold four to six serum bottles.

The incubated serum bottle was allowed to cool to room temperature gradually without interference from turbulent air flow. Then, a 1 ml gas sample was taken from the headspace by using an air-tight syringe with a micro-valve and injected into a GC (Hewlett Packard Model 5710A) which was equipped with an integrator and recorder. The temperatures of the injector, column, and detector were set at 150, 120, and 300 C, respectively. Helium was used as the carrier gas with a flow rate of 20 ml/min. The gas

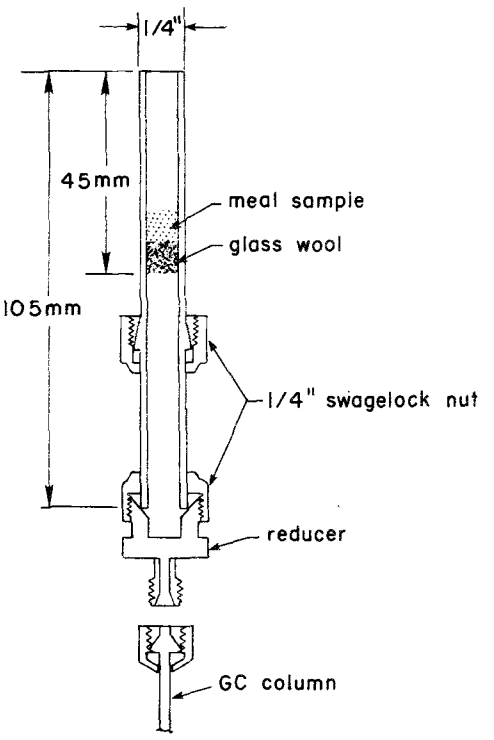

FIG. 2. Cross-sectional diagram of the modified sample adaptor used in the direct GC method.

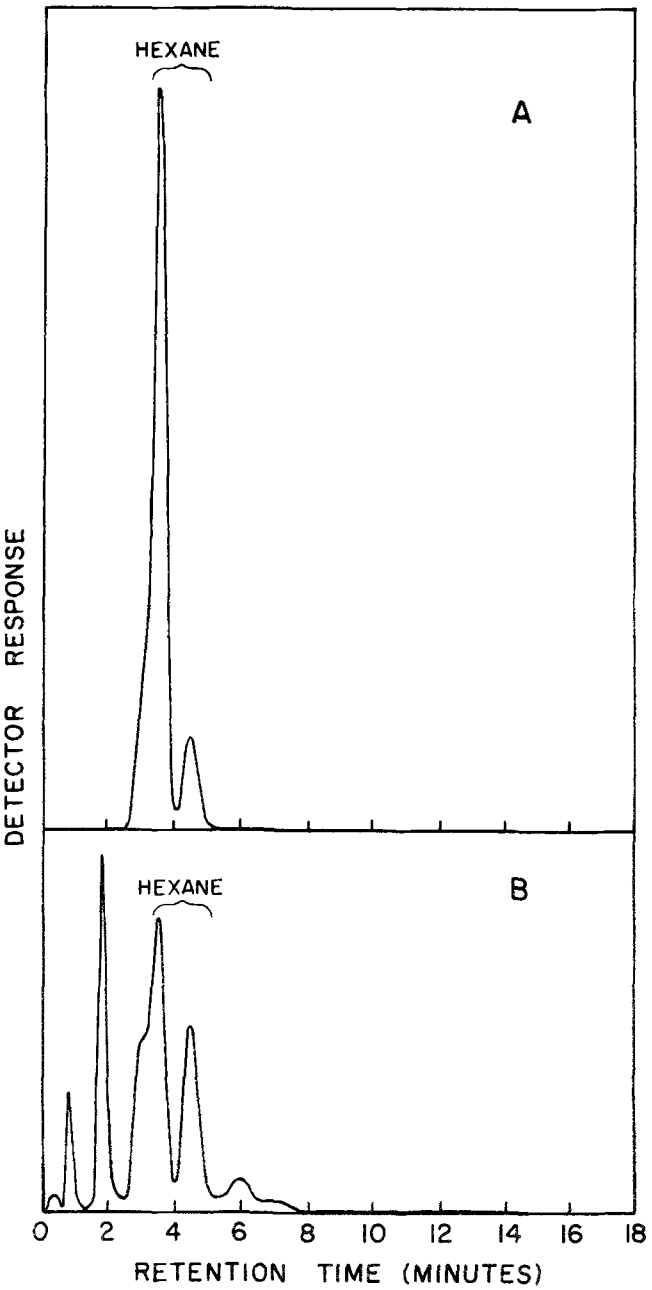

FIG. 3. Gas chromatograms of a commercial hexane (A) and a hexane extracted glandless cottonseed meal (B) by the modified volatilization method.

sample was chromatographed in a 3 ft x 1/8 in. stainless steel column packed with Porapak P (80-100 mesh) which was obtained from Waters Associates, Inc., Milford, MA. In the chromatograph the separated components were detected by a flame ionization detector, and the peak sizes were integrated proportionally by an electronic integrator.

Direct GC Procedure

To compare results, a direct GC technique similar to that described by Fore and Dupuy (2) was also performed. However, a slightly different sample adaptor, as shown in Figure 2, was designed in order to utilize the available GC. A 105 mm x 1/4 in. stainless steel tube, connected with a Swagelock nut and reducer for accepting the GC column, was used as the sample tube in place of the injection port liner which was originally installed in the GC. The sample tube was plugged with glass wool to a depth of 4.5 cm, and a 0.04 g (± 0.0001 g) flour sample was weighed and introduced into the stainless steel sample tube.

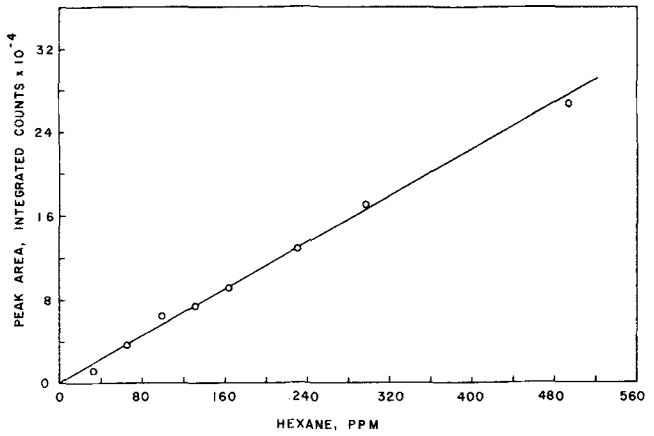

FIG. 4. Calibration curve of hexane in meal for the modified volatilization method.

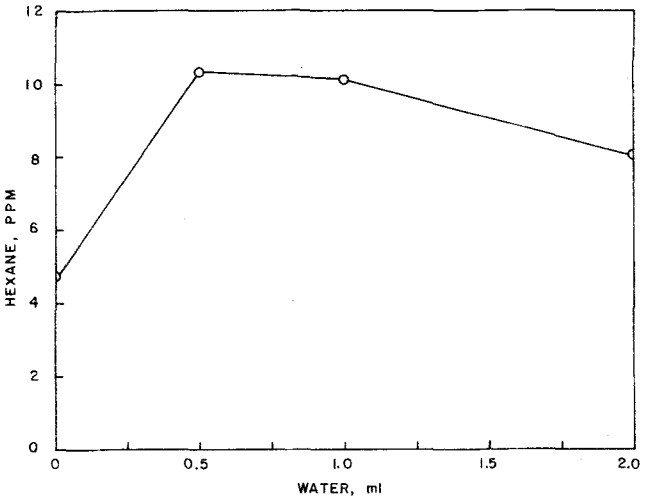

FIG. 5. Effects of the amount of water added on the amount of residual hexane released from 2 g of meal sample.

To prevent loss of hexane from the meal sample, the following steps were used. First, the sample tube was connected to the inlet end of the GC column which was kept at 55 C. Next, the sample tube was inserted into the injection port (set at 100 C). Then, 80 µl of distilled water was injected through a septum on top of the sample meal. After 5 min, a temperature program for the column was started that went from 55 to 120 C, with a heating rate of 32 C/min. The experiment was stopped as soon as the hexane peak was recorded and integrated. In order to make sure that no contaminated components were retained in the GC column, the column was always purged with 20 µl water at 250 C before the next analysis was started.

Preparation of Calibration Curve

The standardization for the modified volatilization method was carried out according to the procedure described earlier. Two grams of screw pressed cottonseed meal were used in the serum bottle into which a known quantity of pure hexane was introduced with a microsyringe. For the direct GC method, an empty serum bottle was used as described by Fore and Dupuy (2) to obtain the calibration curve.

RESULTS AND DISCUSSION

Figure 3 shows typical gas chromatograms of a commercial hexane and hexane extracted cottonseed meal by

TABLE I

Residual Hexane in Glandless Cottonseed Meals (GCSM) Determined by Modified Volatilization and Direct Gas Chromatographic (GC) Method

Method	Sample[a]	Number of replicates	Residual hexane average (ppm)	Average deviation (ppm)	Percentage deviation (ppm)
Modified volatilization	GCSM-A	4	10.3	0.6	6.1
	GCSM-B	3	80.5	1.7	2.1
	GCSM-C	3	83.1	2.6	3.0
	GCSM-D	3	100.3	6.3	6.2
	GCSM-E	8	104.0	4.8	4.6
	GCSM-F	4	295.5	14.5	4.9
	GCSM-G	2	645.0	31.5	4.9
	GCSM-H	4	89,752.0	5095.0	5.7
Direct GC	GCSM-I	3	10.1	0.2	2.0
	GCSM-II	2	18.6	1.1	5.9
	GCSM-III	2	22.8	0.8	3.5
	GCSM-IV	2	47.1	0.7	1.5
	GCSM-V	2	51.6	0.8	1.6
	GCSM-D	3	90.2	13.4	14.8

[a]All samples were produced from hexane extracted glandless cottonseed (GCS) by Food Protein Research and Development Center.

TABLE II

Comparison of Resdiual Hexane in Glandless Cottonseed Meals (GCSM) Obtained from Modified Volatilization and Direct Gas Chromatographic (GC) Method

Sample	Residual hexane, ppm		% Variation
	Modified volatilization	Direct GC	
GCSM-II	25.5	18.6[a]	27.1
GCSM-V	72.3	51.6[a]	28.6
GCSM-D	100.3[b]	90.2[b]	10.1
GCSM-F	295.5[b]	180.0	40.4
GCSM-G	645.0[a]	254.0	60.6
GCSM-1	9,988.0	517.0	94.8
GCSM-H	89,752.0[c]	1,353.0	98.5

[a]Average of two tests.

[b]Average of three tests.

[c]Average of four tests.

the modified volatilization procedure. The calibration curve for the modified volatilization method can be found in Figure 4.

Utilization of water is the key to driving the residual hexane out of the meal. Without water, the residual hexane released from the same sample was only about 50% of that when an optimum amount of water was used. For the modified volatilization method, 0.5 ml of water was found to be the optimum level of water for a 2 g flour sample. The effects of different amounts of water on the degree of hexane released from the same lot of cottonseed meal sample are shown in Figure 5. When excess water was used, 2:1~3:1 (water/flour, v/w), the determined residual hexane dropped as much as 50% to 70% of that obtained by using the optimum amount of water.

From the data listed in Table I, it can be seen that the reproducibilities of both modified volatilization and direct GC methods are reasonably good. The value of percentage deviation was calculated by dividing the average deviation from the means by the arithmetic mean (average) of two or more replicates for each of the samples mentioned. The modified volatilization method appeared to be quite reproducible even at higher hexane levels. On the basis of the limited number of comparison tests between these two methods, as shown in Table II, the residual hexane levels determined by the modified volatilization method are higher than those obtained from the direct GC method. This deviation might be explained by the following reasons. First, there were differences in incubation temperature. Although Fore and Dupuy (2) used a temperature of 105 C in the injection port of their GC, the Hewlett Packard GC does not have temperature settings between 100 and 150 C for the injection port; therefore, a maximum temperature of 100 C was used for the sample adaptor in the direct GC method. The incubation temperature used for the modified volatilization method was 125 C. Second, the direct GC method used a much higher water to meal ratio, 0.08 ml:0.04 g, than the modified volatilization method, 0.5 ml:2 g. Third, there were also differences in the retention time of the water vapor as it permeated the meal sample for the two methods. If the 0.08 ml of water was vaporized immediately after it was introduced into the injection port for the direct GC method, the estimated contact time between water vapor and flour particles would be less than 1 sec for a flow rate of 20 ml/min for the carrier gas. This short contact time might not be long enough to drive most of the residual hexane out of the meal.

The results obtained from this preliminary work have demonstrated that the modified volatilization method can cover a wide range of residual hexane in meal with good reproducibility.

REFERENCES

1. Dupuy, H.P., and S.P. Fore, JAOCS 47:231 (1970).
2. Fore, S.P., and H.P. Dupuy, Ibid. 49:129 (1972).

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