Headspace Gas Analysis of Salad Oils by Mass Spectrometry

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ABSTRACT

A mass spectrometer was used to analyze the content of hydrogen, nitrogen, oxygen, carbon dioxide and argon in headspace gas of commercially packaged soybean, cottonseed and corn salad oils. A leakproof sampling system was designed to avoid air contamination and obtain a representative headspace gas sample. Some edible oils are packaged under pure nitrogen, whereas other samples contained various amounts of oxygen in the headspace gas. The presence or absence of argon in the headspace gas indicates that some oils are packaged with pure nitrogen and others with nitrogen obtained by controlled burning of hydrocarbons to remove all the oxygen in air. The presence of hydrogen in some samples where argon was also present suggested that catalytic purifiers were used to remove the last traces of oxygen and to ensure pure nitrogen for packaging oils. The decrease in oxygen of oils bottled in air was followed during storage at room and at elevated temperatures.

The composition of headspace gas is an important factor in long term storage of packaged salad oils. Salad oils stored under air for 6-10 weeks at 100 F receive lower flavor scores than those stored under nitrogen (1). Oxygen not only dissolved in the oil, but also present in the headspace, reacts with oil to give peroxides and oxidative cleavage products. Many different chemical and physical methods are followed throughout the food industry to determine the oxygen content of packaged foods. Older methods of analysis based on a direct chemical determination of oxygen, such as the Orsat or Winkler techniques (1-4), have been largely superseded by the physical methods of gas chromatography (5-10) and paramagnetic measurement (4) for the determination of oxygen content. In edible oil processing, the polargraphic oxygen electrode has been effective according to several publications (11-14). Initially we explored its use, but the particular electrode reported on by some of these investigators was no longer manufactured in the U.S. Its replacement, a new and considerably changed electrode, has not been satisfactory in our hands for headspace analysis. Consequently we undertook studies using mass spectrometry.

A mass spectrometer resolves all components in a headspace gas mixture with great accuracy and range of concentration, if these elements are desired. Although widely accepted for gas analysis, the application of the mass spectrometer to storage headspace gas analysis of food packages is limited. Mass spectrometry is not a method suitable for control or routine analysis, and this disadvantage limits its use to research or basic investigation in well-defined areas. Detection of leaks or sampling errors is probably more readily ascertained when a mass spectrometer is the analyzing instrument. Drastic or unwarranted changes in the ratio of normal components become obvious when all components of the mixture are determined. Error detection is more difficult when changes in only a single component are measured.

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In this report we present a reliable sampling technique and data on headspace gas analysis of several commercially processed vegetable salad oils packaged in glass bottles.

EXPERIMENTAL PROCEDURE

Salad oils packaged in pint and 1.5 pint screw cap bottles came from retail stores, from warehouses, direct from the processors, and in one plant directly off the bottling line. Upon receipt of all bottles, some were held at 34 F and others were frozen at 0 F until analyzed or placed under accelerated storage conditions. Part of the samples obtained directly from the bottling line were frozen immediately and transported in dry ice. The frozen samples were stored at 0 F and kept frozen until after the headspace gas analyses were completed. Accelerated storage samples were placed in 100 F forced draft air ovens. All samples were stored in the dark.

A gas sampling tool of our own design was constructed from brass stock by the Laboratory's machine shops. Figure 1 is an expanded view drawing showing details of construction. Several of these tools had to be made in order to fit each size of bottle cap. The tool is firmly fastened to the bottle cap by a 1/2 in. hose clamp. The threaded center post of the tool has a small hole drilled in it to receive the hypodermic needle and the bottom end of the post contains a small cup that will hold and compress two silicon rubber septums (gas chromatographic inlet septums) as the post is tightened against the bottle cap. The septum next to the bottle cap has a 3/32 in. hole at its center to allow a space where the hypodermic needle can be positioned to evacuate the bottle gasket and the space around the bottle threads. It is advantageous to use a small amount of silicon stopcock grease on each septum to ensure a vacuum tight seal.

The bottle cap is specially prepared before the sampling tool is attached. The bottom edge of the bottle cap is sealed to the glass with Apiezon wax W-2. The wax and bottle cap are warmed sufficiently with heat lamps to soften (partially liquefy) the wax and it is applied with a 1/8 in. diameter wooden dowel. After a ribbon of wax has been applied completely around the bottle, the wax is worked by hand against the cap and bottle. By dipping the hands in cold water and not having the wax too soft, a good seal is obtained and the wax does not stick to the fingers.

The metal at the exact center of the bottle cap, obtained by marking with a centering square, is ground away with an electric hand grinder. When sufficient metal has been removed from an area about 1/8 in. in diameter, the thin layer remaining can be pierced with a steel pick and the gasket layer exposed. Grinding the metal stops before any gasket material is exposed. The sampling tool is now fastened to the bottle cap, and the bottle positioned under the sampling needle of the mass spectrometer. The bottle is raised on a lab jack until the needle touches the sealing (top) septum. By careful feeling the needle is forced by hand through the top septum.

Vacuum is now applied to the entire system removing all the air from the bottle cap, gasket material, and the inlet system of the mass spectrometer. The system is checked for leaks by a valve, which cuts off the system from the vacuum pump. A system showing no pressure drop on a

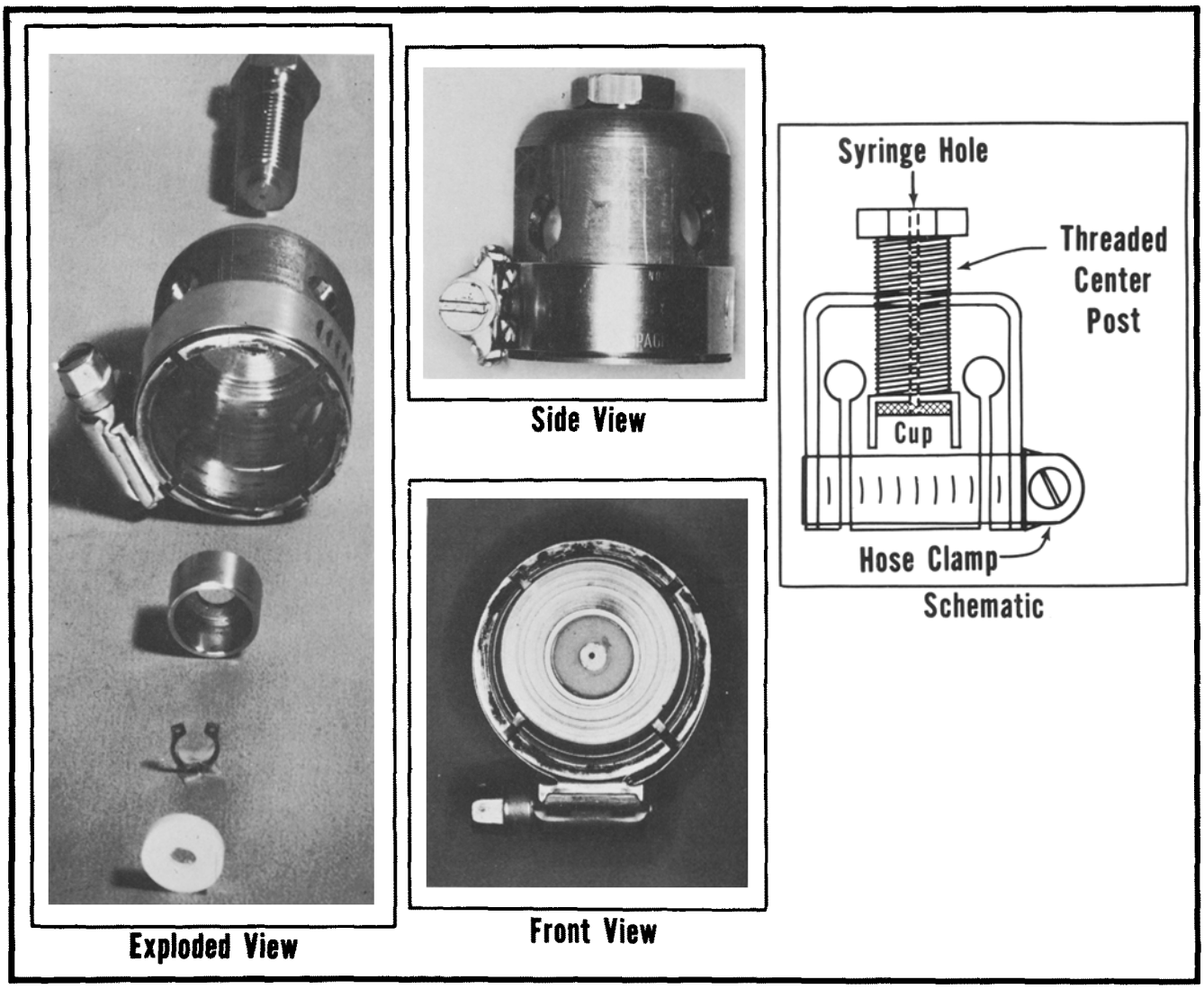

FIG. 1. Expanded view and drawing of headspace gas sampling tool for use on screw cap seals.

mercury manometer within 2 mm is accepted as leak free. Since practically all leaks involve the wax seal, many times by working the wax with the thumb and fingers, the leak can be sealed and the sample saved. The cap liner prevents leakage of the headspace sample into the evacuated system.

When a leak free system has been established, the needle can now be forced through the gasket and through the bottle cap liner. Pint bottles have about 22 ml of free headspace and with our sampling system a pressure of 170-200 mm is obtained from the headspace gas. The pressure in the system is slowly lowered to 100 mm by bleeding off the excess gas and another valve is closed trapping about 10 ml of gas at 100 mm pressure. This 10 ml sample is bled into the mass spectrometer through a gold-foil pin-hole system. After 2 min is allowed for equilibrium to be established, readings from the mass spectrometer scanning system are taken. Figure 2 diagrams the sampling technique and the inlet system to the mass spectrometer.

Mass spectra are corrected for background errors. Peak heights are calculated on the basis of known sensitivities and the appropriate amplification settings of the read-out system. These mass spectra are then normalized to 100% for the gases analyzed. Both background and sensitivity determinations are taken frequently between runs. Sensitivities can be determined from pure gas samples, air or a standard mixture of gases of known composition. Table I records sensitivities from a Bendix Time-of-Flight mass spectrometer for an air sample. From the standard deviations, oxygen would have a relative error of about 7% of the value reported and levels of 0.1% or less could be determined. Sensitivity and accuracy could be greater with instruments having less background and high sensitivity.

RESULTS AND DISCUSSION

Soybean Salad Oils

Hydrogenated winterized soybean salad oils from four different processors were evidently packaged under a variety of conditions. Table II contains headspace analyses of two lots of oil of the same brand name. The results differ surprisingly; one lot (A1-A3) contains an appreciable amount of hydrogen and more argon than that found in air. However both lots of oil have zero oxygen content in the headspace gas. These analyses have been interpreted as resulting from two types of nitrogen used in packaging the oil. In the first samples nitrogen gas was produced by burning hydrocarbons in air to remove the oxygen; this treatment would increase nitrogen and argon contents proportionately. The hydrogen has been purposely introduced into the gas system to remove any oxygen completely by passing the mixed gas system through a catalyst bed to turn the last traces of oxygen to water. Such catalytic purifiers are commonly employed to remove as much as 3% oxygen from many gas systems. The resulting

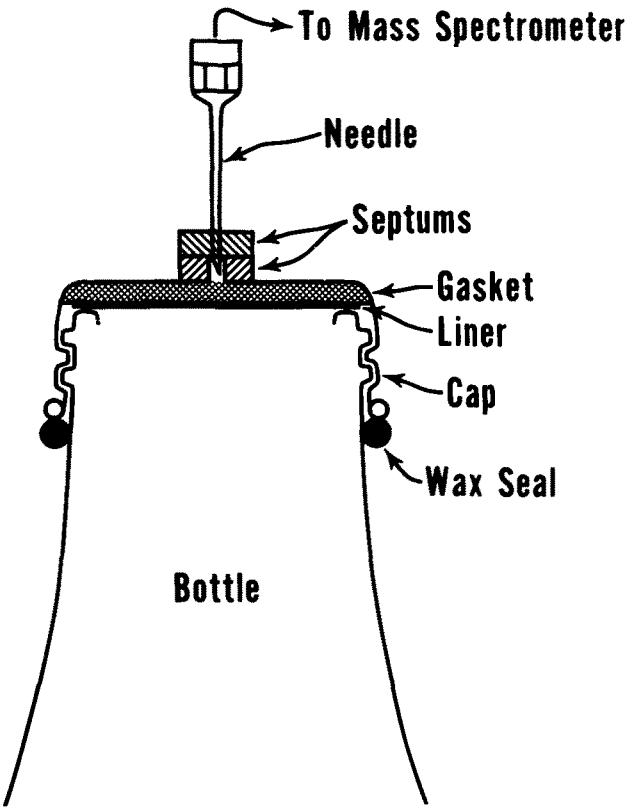

FIG. 2. Drawing of sampling tool showing position of septum seals and wax seal on threads of cap.

gases are reported to be completely free of oxygen (15).

Headspace analysis of the other samples indicate a gas composition like that of pure compressed or liquefied nitrogen containing only a trace of argon. It is assumed that these two systems of nitrogen gas production were employed to give a packaging gas of 0% oxygen content. Long term storage of the A-3 oil (38 weeks, 100 F) did not change the composition of headspace gas because of the complete absence of oxygen and absence of any leakage through the cap.

Table III-A shows analyses of samples apparently packaged under air by two different processors (B and C). The alternate supposition can be made that all samples were "leakers" and that the headspace gas we analyzed was air. The percentage of argon was constant and equal to that of air. The oxygen content was slightly less than air, as would be expected if any reaction of the oxygen with the oil had taken place. The age of these samples at the time of analysis was unknown but assumed to be typical, as both lots of oil

TABLE I

Mass Spectrometric Sensitivities (From Air)

Gas	Peak height/ mm gas pressure	Standard deviation[a]	Air composition,[b] %
CO_2	28.4×10^3	4.0×10^3	0.033
A	5.0×10^3	0.16×10^3	0.934
O_2	2.8×10^3	0.19×10^3	20.946
N_2	4.1×10^3	0.14×10^3	78.084
H_2O	5.0×10^3	0.5×10^3	(2.2 at 25 C and 71% RH)

[a]N for oxygen and nitrogen was 14; for other components, it was 6.

[b]Source: Handbook of Chemistry and Physics, 49th ed., Chemical Rubber Co., Cleveland, Ohio, 1968-69.

TABLE II

Headspace Gas Analysis of Soybean Salad Oils Indicating Different Sources of Nitrogen

Samples	O_2, %	N_2, %	A, %	H_2, %
A-1	---	95.4	1.6	2.8
A-2	---	93.8	1.7	4.4
A-3 (38 weeks, 100 F)	Trace	94.5	1.6	3.3
A-4	Trace	99.8	0.028	0.001
A-5	---	99.9	0.031	0.04

TABLE III

Headspace Gas Analysis of Bottled Vegetable Oils

Type oil		Gas composition, %				
		CO_2	A	O_2	N_2	H_2O
A. Hydrogenated winterized soybean oils[a]	1B	0.034	0.93	18.2	80.5	0.38
	2B	0.40	0.94	16.9	82.3	0.25
	1C	0.49	0.95	20.2	78.4	0.45
	2C	0.33	1.08	17.2	81.4	0.30
B. Soybean salad oils[b]	1D	0.021	0.39	9.1	90.2	0.30
	2D[c]	0.003	0.11	2.4	97.0	0.45
	3D	0.035	0.84	19.8	79.1	0.25
	4D[c]	0.004	0.15	2.1	97.4	0.36
C. Cottonseed salad oils[d]	1E	0.001	0.054	00.0	99.8	0.12
	2E	0.001	0.044	0.0	99.9	0.09
	1F	0.034	0.74	6.0	92.5	0.17
	2F	0.035	1.1	5.1	93.6	0.06
D. Corn salad oils[e]	1	0.010	0.34	3.3	96.7	0.04
	2	0.003	0.31	0.11	99.3	0.28
	3	0.006	0.32	1.2	98.2	0.25
	4	0.004	0.23	0.41	98.9	0.42
	5	0.002	0.29	0.18	99.8	0.28
	6	0.006	0.26	2.09	97.9	0.24

[a]Samples from commercial warehouses.

[b]Samples shipped by processor to Northern Laboratory.

[c]Partially hydrogenated winterized soybean oil. Samples 2D and 4D are averages, see text.

[d]Shipped by two processors to Northern Laboratory; frozen and held at 0 C until analyzed.

[e]Local purchase.

TABLE IV

Headspace Gas Analysis of Bottling Line Corn Oil Samples Held at Room Temperature

Gas	6 Hr at room temperature, %		1 Day at room temperature,[a] %	
CO_2	0.81	0.011	0.012	0.012
A	0.35	0.30	0.25	0.25
O_2	9.1	8.7	6.8	6.9
N_2	89.9	90.3	92.7	92.2
H_2O	0.64	0.76	0.24	0.63

[a]Held overnight at 34 F.

came from a commercial warehouse. Indications are that these samples were saturated with air at the time of bottling. Had the oil been protected by pure nitrogen up to the time of bottling, it would be saturated with nitrogen but have no dissolved oxygen. If oil that had been nitrogen-protected up to the stage of bottling and capping that was done in air, then the level of oxygen in the headspace gas would be below that found in air.

Samples of liquid salad soybean and hydrogenated winterized salad oils were supplied by processor D from a plant in which test runs on nitrogen packing were underway. The headspace analysis of these samples is given in Table III-B. Details of the processing were not made available, but the average results made at the time samples were received, shown under 2D and 4D, indicate a final composition having a slight amount of air contamination. Air contamination can be inferred both from the oxygen content and the ratio of the argon to oxygen, which ratio is the same as found in air. Results shown under 1D and 3D are from the same lot of oil, but these bottles had been stored, frozen and moved as a lot sequentially into three different cold rooms. It is not believed that the samples thawed during any of these moves, but the last cold room was at -24 F, and the previous one was at 0 F. This cycling and extremes in temperature change, or both, caused the caps to leak and a variable amount of air entered the headspace. The size, design, type of metal, type of gasket material and liner vary with each processor's bottle. These results, plus data on samples stored in dry ice to be discussed later, indicate that headspace analysis for frozen samples held at extremely low temperature is liable to be erroneous because of leaks. Caps are loosened by extreme changes in temperature and leaks develop.

Cottonseed Salad Oil

Two processors shipped cottonseed oil directly to the Northern Laboratory. The analyses of four individual bottles are shown in Table III-C. Samples 1E and 2E from a fifth processor indicate that these bottles were capped under pure nitrogen. These analyses agree with those for A-4 and A-5 samples in Table II, and the analysis of nitrogen gas agrees with that from commercial supplies of liquid nitrogen. Headspace analysis of samples 1F and 2F from the sixth processor would indicate a packaging process in which the oil is protected with nitrogen up to the time of bottling, and then the bottling and capping operation was probably done in air. Under such conditions of operation the level of oxygen will be lower than that found in air.

Corn Oil Studies

Several corn oil processors are within the region of the Northern Laboratory, and because samples directly from the bottling line were easily available, as well as from local retail markets, a number of headspace investigations were undertaken with this oil. Corn oil from the particular plant from which all samples came is protected by nitrogen throughout refining and processing operations. The oil is bottled by pulling a partial vacuum on the bottle, allowing the oil to enter the bottle under vacuum, and only after the vacuum is released from the filler is the oil exposed to air. The headspace volume is about 22 ml. This amount of air will come to equilibrium with essentially pure nitrogen released from the nitrogen-saturated oil with which the bottle was filled. The final oxygen level in headspace of the bottle will depend upon the equilibrium of dissolved oxygen, plus any that is reacted with the oil or container wherever metal cans are used.

Table III-D shows the results of headspace analysis of six bottles of corn oil purchased on the Peoria retail market. Although oxygen levels vary, they are considerably below what might be expected for an air-bottled sample. The highest level of 3.3% and the lowest of 0.11% indicated that the bottles are not leakers, and because of difference in oxygen content it is concluded that oxygen has reacted with the oil. If only absorption had taken place, all oxygen levels should be identical, as can be seen for the level of argon.

To gain information on the absorption equilibrium oxygen level, samples of corn oil were retrieved directly from the bottling line. None of the samples for which analytical results are shown in Tables III-D to V were ever frozen. One-half of the samples acquired, however, were frozen immediately in dry ice. Six hours after the samples were bottled, the headspace gas analysis was performed on two unfrozen samples. Table IV shows these analyses plus

TABLE V

Headspace Gas Analysis of Corn Oil Samples Stored at 100 F

Gas	1 Week, %		2 Weeks, %		3 Weeks, %	
CO_2	0.003	0.003	0.001	0.001	0.001	0.002
A	0.17	0.15	0.18	0.17	0.18	0.21
O_2	1.4	0.94	0.81	0.03	0.0	0.0
N_2	98.5	98.7	98.9	99.4	99.6	99.5
H_2O	0.09	0.15	0.30	0.42	0.37	0.26

TABLE VI

Headspace Gas Analysis of Bottling Line Corn Oil Samples Held Frozen

Gas	1 Day in dry ice, %		1 Week at 0 F, %		2 Weeks at 0 F, %	3 Weeks at 0 F, %
CO_2	8.4	6.75	0.008	0.024	0.018	0.012
A	0.74	0.69	0.23	0.32	0.48	0.26
O_2	20.2	17.4	6.3	6.4	10.4	5.8
N_2	70.5	74.9	93.3	92.9	88.8	93.7
H_2O	0.24	0.26	0.23	0.37	0.33	0.17

those made 1 day after bottling. The analysis of the first sample reveals a slight leakage of the cap. This leakage is indicated primarily because of increased carbon dioxide, probably introduced during transit and abetted by slight cooling of the bottles, The analysis of the second sample can be accepted as fairly accurate and typical since the levels of argon and carbon dioxide agree with later analysis. The 1-day room-temperature samples show good agreement and indicate no leakage; argon levels are about the same as the locally purchased samples. Such levels of oxygen and nitrogen might approximate the absorption equilibrium level of the headspace gases before any reaction with oxygen has taken place.

The loss of oxygen from the headspace gas is rather rapid for corn oil under storage at 100 F. Results of storage for 1, 2 and 3 weeks are summarized in Table V. Argon equilibrium appears to be about 0.19% for 100 F against the value of 0.25% for samples held at room temperature. Oxygen level drops from an initial value of approximately 7 to 1.5% within a week, to less than 1% at the end of the second week, and to zero within 3 weeks. Oxygen values for individual bottles at the end of the second week's storage were 0.81, 0.17 and 0.03%, which shows some bottle-to-bottle variation as might be expected.

Table VI presents headspace analyses of samples that were frozen in dry ice as they were removed from the bottling line. Both samples held 1 day in dry ice before analysis are considered leakers, since both show high levels of oxygen and carbon dioxide. The samples analyzed after 1, 2 and 3 weeks were also frozen in dry ice while being transported. After 1 day in dry ice they were removed and stored frozen in a 0 F cold room. Samples were also kept frozen while the headspace analysis was performed. These headspace gases represent a mixture, which probably results from dilution of air trapped when the bottle was capped, by nitrogen forced from solution in the oil when the fat was frozen. The pressure inside the bottles of the frozen samples was beyond the range of the manometer in our system. We have no information on the dilution except what can be calculated from the known value of 22 ml of air when the bottle was capped. To reduce the oxygen content to approximately 6% requires a 3.5-fold dilution. The amount of argon present would also indicate this level of dilution. Results for the 2 and 3 week 0 F storage samples are from single bottle analysis and do not indicate any leakage. Screw cap seals are prone to leak when bottles are cooled to sub zero temperatures.

The wide range of oxygen levels found in the headspace gas of bottled vegetable salad oils indicates that the degree of inert packaging varies from total protection, through partial to no protection. Salad oils capped in air indicated absorption of oxygen within hours. Oils under storage at 100 F show high absorption within a few days, and the entire amount of oxygen in the headspace gas was consumed within 2-3 weeks' storage.

The mass spectrometric method gives true identification of gases, is adaptable to other canned products, is usable for the development of leak free sampling systems, is sensitive and should be useful in long term storage and organoleptic studies of various foods.

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