Determination of Isopropyl Alcohol in Solid Fish Protein Concentrate by Gas-Liquid Chromatography

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A method for the determination of isopropyl alcohol in fish protein concentrate (FPC) by gas-liquid chromatography consists of heating the FPC to 180° C. in a closed tube for 20 minutes to release the isopropyl alcohol and then injecting the alcohol directly into the system. The method is also applicable to the determination of similar volatile components in other solid materials.

Recently, the U. S. Food and Drug Administration approved the manufacture and sale in the United States of fish protein concentrate (FPC) (Bureau of Commercial Fisheries, 1966) as a food additive. The FPC is made by either of two solvent extraction processes. One process, developed in this laboratory, involves the use of isopropyl alcohol as the extraction solvent. To maintain quality control of desolventization and to ensure that the limits on residual solvent content imposed by the FDA are met, it is necessary to measure the amount of isopropyl alcohol remaining in the final product. Furthermore, in the course of development of food products incorporating FPC, it is often necessary to measure the amount of solvent remaining in the processed foodstuff.

Conventional methods for the determination of alcohol require either making derivatives of the alcohol (Holley and Holley, 1952; Sabetay, 1939) or extracting the alcohol (Ackman *et al.*, 1967; Hornstein *et al.*, 1960; McCreadie and Williams, 1957) prior to analysis, all tedious chemical procedures. The first approach has the disadvantage of not determining the alcohol directly, and in all approaches the actual fraction of the alcohol removed from the sample is often difficult to determine.

The use of gas-liquid chromatography for the direct determination of alcohols in liquids has been described by several workers (Bodnar and Mayeux, 1958; Demick and Corse, 1965; Fox, 1958; Neigisch and Stahl, 1956; Parker *et al.*, 1962; Rhodes, 1958; Rogozinski *et al.*, 1963; Smith, 1959). However, very little information has appeared on the use of the gas-liquid chromatograph (GLC) for the determination of alcohols in solid samples. McCreadie and Williams (1957) developed a GLC method for the determination of certain volatile components in solid samples, but did not describe application to alcohols.

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Although GLC determinations were most likely to fit the authors' requirements, none of the published methods was conveniently adaptable to a rapid simple method for the determination of isopropyl alcohol in FPC. The objective of this work, therefore, was the development of a rapid and accurate GLC method for determining isopropyl alcohol in solid samples.

EXPERIMENTAL TECHNIQUES

Detection and Measurement of Isopropyl Alcohol. The GLC employed was the F and M Biomedical 400 equipped with a hydrogen flame ionization detector. A stainless steel column (6 feet × $^1/_4$ inch o.d. × $^3/_{16}$ inch i.d.) was used, packed with 40% Castorwax on 60- to 80-mesh Chromosorb W (acid-washed). This packing, suggested by Parker *et al.* (1962), was chosen because its properties provide good separation of isopropyl alcohol from other volatiles.

Optimum Conditions for Detection of Isopropyl Alcohol. The detection of isopropyl alcohol and the separation of its response from those of acetone and ethyl alcohol were found to be accomplished best with a column temperature of 80° C., carrier (He) gas flow of 15 ml. per minute, flash heater and detector temperatures of 180° and 200° C., respectively, and H_2 and air flow rates of 20 and 425 ml. per minute, respectively. (The flash heater temperature is not critical for calibration of standards as long as it is above their boiling points.)

To determine the conditions listed, water solutions containing varying concentrations of isopropyl alcohol, acetone, and ethyl alcohol were used. Ethyl alcohol was added to isopropyl alcohol because it has a retention time similar to that of isopropyl alcohol with the column packing used and because foodstuffs containing FPC may also contain small amounts of ethyl alcohol. Acetone was added because of the possibility of oxidation of isopropyl alcohol to acetone during the preparation of FPC or

foodstuffs containing FPC. The optimum conditions for the detection of isopropyl alcohol gave the best compromise between sensitivity and resolution and minimize the possible effect of water on the response.

DETECTOR RESPONSE AS A FUNCTION OF LIQUID SAMPLE SIZE. The linearity of the response throughout the concentration range was established by measuring peak height as a function of concentration of isopropyl alcohol with a series of 1-μl. samples of a water solution of isopropyl alcohol of different concentrations. With a total attenuation of 3200 the response was linear over a 12-fold concentration range. At this attenuation, full-scale deflection corresponds to 0.16 mg. of isopropyl alcohol, which means that detection and measurement of 1 μg. of isopropyl alcohol are well within the capabilities of the apparatus.

REPRODUCIBILITY OF INJECTION OF KNOWN QUANTITIES OF LIQUID SAMPLE. To determine the reproducibility of injection, six 1-μl. samples of 10% isopropyl alcohol in water were added to the column on two consecutive days, and the data obtained were analyzed statistically. The maximum standard deviation for the mean was ±2%.

Adaptation of Method to Solid Sample Analysis. Although the F and M Biomedical 400 GLC was not intended for use with solid samples (samples are injected along a vertical axis), the machine was adapted to accept solid samples of FPC with the use of the F and M solid sample holder. With this holder, a sample is sealed inside a standard melting point tube, which is eventually crushed inside the system. Because of the vertical arrangement of sample holder and column, particles of glass and sample are deposited on the glass wool plug in the top of the column; hence this material had to be removed after each run. The stainless steel column can be removed, cleaned, and replaced after each run with no danger of breakage. (Cleaning consists simply of inverting the column and tapping out the particles of glass and sample deposited on the plug. This can be done in 1 to 2 minutes. Occasionally, it is necessary to change the glass wool plug.) Furthermore, the relatively large inside diameter of the metal column accommodated the sample holder with ample annular clearance and no impediment to gas flow. With the particular apparatus used, 10-mg. samples of ground FPC are weighed in 5-cm. melting point tubes. This combination allows all the sample to be within the flash heater and provides enough room to seal the end of the tube without heating the sample. The filled tube is then placed in the flash heater.

FLASH HEATER TEMPERATURE. To determine optimum conditions for liberating the isopropyl alcohol, samples of a given FPC were heated in the flash heater for 15 to 60 minutes at temperatures from 100° to 310° C. No change in response at the retention time for isopropyl alcohol was noted with heating time greater than 15 minutes, and 20 minutes was chosen as the standard time for routine analysis. Much of the experimental work was carried out at 30 minutes as further assurance of complete isopropyl alcohol liberation. The response increased with rising temperature, however, up to 180° C., after which no further increase was noted up to 310° C. At this temperature, the sample was so decomposed that it was difficult to crush the glass tube to inject the volatile components into the gas stream. Consequently, the highest temperature at which a measurement was made was 280° C., at which temperature, after 30 minutes, the sample was almost completely charred. The likelihood was considered small that enough of the original molecular structure remained to retain significant amounts of isopropyl alcohol.

At the highest temperature used, some response was noted at retention times lower than that for isopropyl alcohol. However, the unchanged isopropyl alcohol response indicated that these peaks were probably caused by some other components in the FPC. Previous work on the thermal stability of isopropyl alcohol (Barnard, 1960; Herndon and Reid, 1928) has established that no significant decomposition will occur after 20 minutes at 180° C., which is the standard condition chosen for heating the sample.

Interferences from Other Volatile Products. The possibility that other volatile products from the heated FPC might be contributing to the isopropyl alcohol peak was also investigated by analyzing a batch of FPC prepared with ethyl alcohol instead of isopropyl alcohol. This material, when heated to 280° C. for 30 minutes, produced a few unidentified peaks with retention times lower than that of isopropyl alcohol, but produced no response at the isopropyl alcohol retention time.

A small peak occasionally appeared before the isopropyl alcohol peak when samples were analyzed under the standard conditions. However, this peak occurred when the melting point tubes were sealed with a gas-air flame instead of a gas-oxygen flame and consequently was assumed to be caused by combustion products in the flame.

DETECTOR RESPONSE AS A FUNCTION OF SOLID SAMPLE SIZE. Samples (4, 8, and 10 mg.) of FPC were analyzed in duplicate. The results showed a linear detector response within the range tested. This linearity eliminates the possibility that the free volume above the sample, or readsorption of isopropyl alcohol by the FPC deposited on the column during the analysis, might affect the response.

Evaluation of Final Method. PRECISION OF METHOD. The precision of the method was tested using three types of samples: an FPC containing a high concentration of isopropyl alcohol, one with a low concentration, and a food mix containing FPC. For each sample six analyses were performed on two consecutive days, and results were statistically analyzed for standard deviations and standard errors (Table I). Undoubtedly, a great part of the variation can be attributed to the inhomogeneity of the material

Table I. Precision of Methods for the Determination of Isopropyl Alcohol in FPC and Food Mix

	Level		
IPA	FPC, %	FPC, p.p.m.	Food mix, p.p.m.
Day-1 mean	2.48	130.8	85.0
Std. dev.	0.1483	14.5384	6.6332
Std. error	0.0606	5.9365	2.7085
Day-2 mean	2.32	114.3	85.0
Std. dev.	0.1049	14.4729	5.4558
Std. error	0.0796	5.9097	2.2278

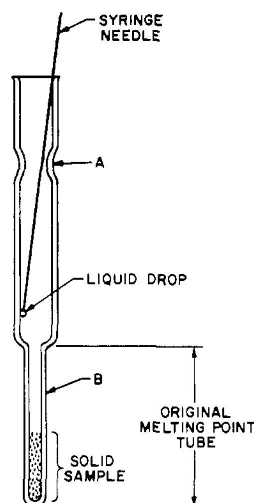

Figure 1. Modified sample tube

being sampled, since only 10-mg. samples are removed from a powder with a broad distribution of particle sizes of several components.

ACCURACY OF METHOD. To determine the accuracy of the method by the usual procedure of adding the volatile component to the sample (in this case isopropyl alcohol to FPC) and measuring its recovery by the analytical procedure proposed presents two major problems. The first is achieving uniform distribution of the alcohol in the solid, without loss. This is a problem because the amounts of isopropyl alcohol to be measured vary from less than 100 p.p.m. to about 2% by weight in dry FPC. For a 10-mg. sample this corresponds to amounts of alcohol from less than 0.00126 to about 0.25 μl. One solution to this dilemma is to add microliter quantities of a water solution of the alcohol and this procedure was investigated.

Attempts to inject 1 μl. of a water solution of alcohol into the melting point tube containing the sample were soon abandoned because the inside diameter of the tube, ca. 2 mm., is too small to accommodate the liquid drop from the syringe needle without a large fraction of the liquid being withdrawn with the needle. To avoid this problem of capillarity, a procedure was devised which involved distillation of the water solution into the melting point tube. To accomplish this, the melting point tube was modified as in Figure 1.

PROCEDURE. A sample (ca. 10 mg.) of FPC prepared with ethyl alcohol was placed in the weighed modified sample tube and tapped down into the melting point tube. The inner surfaces of the tube above the sample level were wiped clean of particles with a cellulose wipe to avoid interference with subsequent sealing of the glass, and the weight of the filled tube was determined.

The syringe needle was inserted into the modified tube and 1 μl. of a solution of isopropyl alcohol in water was injected as shown, in such a way that the drop clung to the side of the tube and did not wet the needle. The needle was then withdrawn.

The tube was quickly sealed at constriction *A*.

The narrow part of the tube (the original melting point tube) containing the solid sample was then immersed in liquid nitrogen, and the upper part containing the liquid drop was gently heated until all the liquid had distilled into the lower portion. Then, while the lower portion was still immersed in liquid nitrogen, the tube was sealed off at *B*, with the same final length as normally used in the GLC.

The entire procedure was repeated with two empty tubes for recovery controls. When the tube and its contents were allowed to come to room temperature it was obvious, from the darkening of the upper millimeter or so of the solid sample, that the liquid had come in contact only with the upper portion of the solid. Consequently, the samples were equilibrated at 100° C. for 63 hours in an effort to make the distribution uniform. However, at the end of 63 hours no change in appearance of the solid samples was observed. The upper dark portion remained dark. Furthermore, attempts at mechanical mixing by agitating the solid within the tube were totally unsuccessful. No amount of tapping or mechanical agitation before or after heating at 100° C. dislodged the dark upper section of the sample which had become an effective plug when originally wetted with the 1-μl. liquid sample. Nevertheless, after "equilibration," each sample tube was inserted into the GLC and heated according to the procedure described above. However, these tubes could not be crushed properly because of the plug, and it was not possible to inject the vapor contents into the carrier gas stream in a sufficiently short time to make the measurement results meaningful.

The action of the water solution on the surface of the powdered sample was not wholly unexpected, since approximately 5% of the FPC is water-soluble. Thus, material was dissolved from the particles which first came in contact with the water and redeposited—in whatever form—in the course of absorption of the water. One consequence of this dissolution-redeposition was particles o completely different surface characteristics from the original particles. Furthermore, desolventizing experiments have shown that water molecules easily replace adsorbed isopropyl alcohol molecules, so the use of water as a carrier would guarantee that the added alcohol would not be bound in the same way as the original alcohol.

This last point brings up the second and by far the most important of the two problems mentioned above. Even if practical means could be found for adding the required quantities of alcohol to the powder, without loss and with uniform distribution, interpretation of recovery results would be open to serious question because one would first have to ascertain that the added alcohol was bound to molecular adsorption sites in the same way as the residual alcohol. Normally, one would add the material to be measured to the solid in question, in this case isopropyl alcohol to FPC made with isopropyl alcohol. The use of water as a carrier, however, would also release some of the residual alcohol, besides preventing the added alcohol from being bound as the residual alcohol was. The release of the residual solvent by the water could be eliminated as a problem by using FPC made with ethyl alcohol, for example, which was used in the experiment described above. However, the dissolution-redeposition effects of the water still prevented proper injection of the sample, besides casting doubt on interpretation of recovery results. True, one could use some other liquid as carrier for the solvent in question—for example, a solution of isopropyl alcohol in ethanol—in which case the complications caused by water

solubility of the solids could be eliminated. However, one would still have to determine whether the solvent—e.g., ethanol—is preferentially adsorbed to the solute—e.g., isopropyl alcohol—or *vice versa*, before any recovery results would be meaningful.

In spite of these problems, a further attempt was made to obtain conventional recovery data, by a technique which avoided liquid contact with the solid sample. Samples of FPC of 10 mg. each (containing either small or immeasurable amounts of IPA) were placed in melting point tubes and a small cellulose packing was placed in each tube, sufficiently above the sample not to be in contact with it, and sufficiently below the top of the tube not to interfere with subsequent sealing of the glass. A solution of 0.01% IPA in water was then injected into each tube directly onto the packing. The tubes were immediately sealed, heated to 100°C. for 2 hours, and then allowed to stand at room temperature for 2 days before analysis. In this way it was hoped that the IPA could be added to the sample solely from the vapor phase and problems associated with water contact could be avoided. The recoveries obtained are listed in Table II.

Because of the problems associated with recovery experiments discussed, and because even the value of the results shown in Table II is open to question, since there is no way of determining the distribution of the added alcohol between the cellulose packing and the FPC sample, the authors preferred to abandon attempts to obtain conventional recovery information and to evaluate the accuracy of the method by the following two criteria: the degree to which the residual solvent molecules are liberated from the substrate, and the degree to which the liberated solvent molecules are unchanged chemically by decomposition or reaction during their residence in the flash heater.

Since no additional isopropyl alcohol could be detected when the sample was heated to charring at 280° C., it seems reasonable to conclude that the liberation of the isopropyl alcohol molecules from the solid FPC must be essentially 100% complete under the operating conditions chosen. Furthermore, since both this investigation and previous work have demonstrated that no measurable decomposition of the isopropyl alcohol occurs under the conditions chosen for analysis, one must conclude that the method described measures essentially 100% of the residual isopropyl alcohol in the solid substrate.

Evaluation of Method Using Other Solids. Wet products, such as fresh bread, soups, gruels, and fish solids from various stages of the extraction, have been analyzed for ethyl and isopropyl alcohols by a combination of vacuum distillation and the technique described in this paper. In these cases, the wet product is distilled under vacuum and the distillate is collected in a trap cooled by liquid nitrogen. (In some cases it is desirable to add some water to the trap prior to distillation, to supply enough liquid to act as a carrier.) This liquid distillate is weighed and analyzed by GLC. The dried solid is weighed, finely ground, and analyzed by the technique described, and the two results are then added after proper weighting.

Isopropyl alcohol has also been determined in dry foods containing FPC, such as cookies, crackers, noodles, and stale bread by this method with satisfactory results. Foods containing varying amounts of FPC have isopropyl alcohol concentrations which vary directly and reproducibly in proportion to the FPC concentration.

Table II. Recovery of Isopropyl Alcohol Added to FPC

Sample	Present, P.P.M.	Added, P.P.M.	Recovered,[a] P.P.M.	% Recovered
1	Nondetectable	80	82	102
2	57	80	141	103
3	54	80	147	110
4	Nondetectable	80	70	88
5	Nondetectable	80	69	87

[a] Average of 2 analyses for each sample.

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