Determination of Trace Sulfur in Fat by Combustion and Reduction of Sulfate to Hydrogen Sulfide

E. L. Pippen and E. P. Mecchi

A method for estimating total sulfur in poultry fat in the range 0 to 10 μg. of sulfur per gram of fat and having the capability of distinguishing between samples differing by less than 1 μg. of sulfur per gram of fat was required during flavor investigations. Fat samples (5 to 15 grams) were burned in about 1 hour at 1000° C. in a vertical quartz tube containing vanadium pentoxide catalyst. Combustion products were passed through peroxide to convert sulfur dioxide to sulfate. High sensitivity and almost complete specificity for sulfur were attained by reducing the sulfate, with hydriodic and phosphorous acids in acetic acid, to hydrogen sulfide, which was determined by the methylene blue method. Distinction between samples which differed by only 0.5 μg. of sulfur per gram of fat was readily achieved. Recovery of sulfur added to fat in the range 0.5 to 2 μg. of sulfur per gram of fat was 73 to 90%.

In recent studies on poultry flavor (Pippen *et al.*, 1969), we needed a method for determining total sulfur in poultry fat in the range 0 to 10 p.p.m. Few, if any, of the many methods for determining sulfur in organic materials (Heinrich *et al.*, 1961) seem to have been applied to the analysis of fat. On the other hand, the problem of analyzing for trace sulfur in petroleum products has received considerable attention and the problems encountered and methods used have been reviewed (Franks and Gilpin, 1962; Milner, 1963). These reviews suggest that traces of sulfur in petroleum are probably most often determined by combustion of the sample in a stream of oxygen or air, following which the sulfur oxides are absorbed and converted to sulfuric acid in hydrogen peroxide solution, and the sulfate is then determined in one of a variety of ways. Therefore, we used this approach for the analysis of trace sulfur in fat.

The problem of cleanly burning macro-sized fat samples (5 to 15 grams) in a reasonable length of time was solved by adopting and modifying the vertical quartz combustion tube procedures of Wilson and Straw (1950), Hudy and Mair (1955), and Hudy and Dunn (1957).

The small amount of sulfuric acid formed in the combustion of our fat samples required that the method for determining sulfate be sensitive to as little as 1 to 5 μg. of sulfur. Furthermore, any method used for the determination of this sulfate must recognize and account for the possibility of interference from other ions, such as nitrate and phosphate, that could be formed during combustion of the fat. These requirements virtually eliminated use of the usual volumetric and gravimetric methods for determining sulfate and also make of doubtful value methods tailored for determining small amounts of sulfate, such as conductometric methods (Hudy and Mair, 1955; Polsky *et al.*, 1947), barium chloranilate methods (Bertolacini and Barney, 1957; Lloyd, 1959; Lysyj and Zarembo, 1959; Spencer, 1960; Stoffyn and Keane, 1964), and a thorium borate–amaranth method (Lambert *et al.*, 1955). However, Gustafsson (1960a,b) and Fildes and Kirsten (1965) have shown that sulfate can be quantitatively reduced to hydrogen sulfide, which can then be determined by the well known highly sensitive and specific methylene blue method. Gustafsson's (1960a,b) method was therefore adapted to and used for the determination of the sulfate formed in the combustion of our fat samples.

Western Regional Research Laboratory, Agricultural Research Service, U. S. Department of Agriculture, Albany, Calif. 94710.

EXPERIMENTAL

Apparatus. COMBUSTION AND SULFUR DIOXIDE ABSORBING APPARATUS. The assembled apparatus (Figure 1) is a modification of that described by Hudy and Mair (1955) and Hudy and Dunn (1957). The fritted glass bubblers they specified in the absorbers were replaced by pierced glass bulbs because the former caused excessive peroxide foaming. Excellent

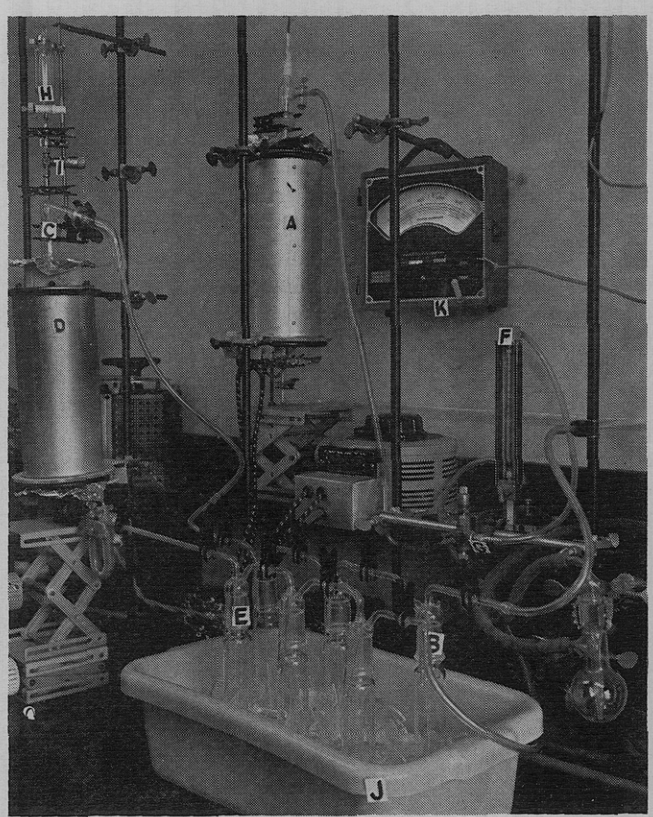

Figure 1. Combustion apparatus

A, Furnace in air purification train
B, Hydrogen peroxide bubblers in air purification train
C, Combustion head
D, Sample combustion furnace
E, Hydrogen peroxide bubblers to absorb SO_2 from sample combustion and to oxidize it to SO_4
F, Flowmeter
G, Air flow regulating valve
H, Dropping funnel
I, Metering valve
J, Ice bath
K, Pyrometer

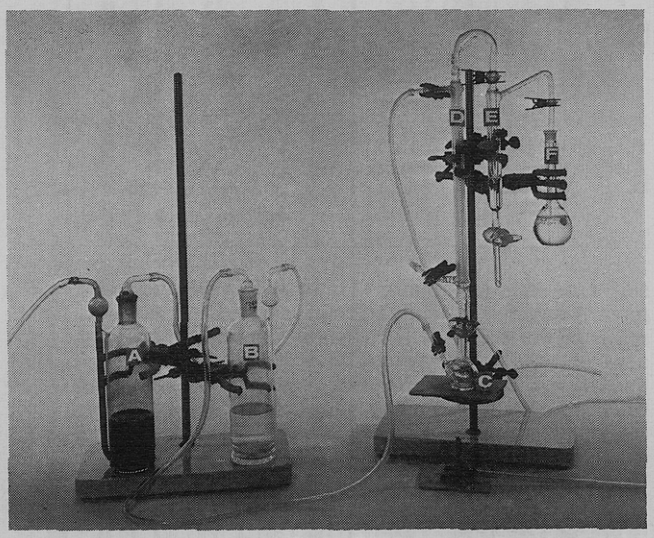

Figure 2. Sulfate reducing and hydrogen sulfide absorbing apparatus

A, Nitrogen scrubber containing potassium permanganate and mercuric chloride
B, Nitrogen scrubber containing water
C, Reducing flask
D, Reflux condenser
E, Gas washing column
F, Hydrogen sulfide absorber

control over the flow rate of fat into the furnace was obtained by using a Nupro series "S" straight pattern fine metering valve equipped with a vernier handle and 1/8-inch Swagelok stainless steel fittings carrying 12/5 stainless steel ball and socket joints (Figure 1, *I*). The fat sample holder (Figure 1, *H*) consisted of a 25-ml. dropping funnel equipped with a Teflon stopcock with integral needle valve. A Schwartz U-tube partly filled with quartz chips was placed between the exit of the combustion furnace and the sample absorbers to condense and retain the small amounts of catalyst carried out of the furnace during combustion. The quartz combustion tubes were made from 31-mm. O.D. tubing with a 35/25 quartz socket joint at the top and a 12/5 quartz ball joint at the bottom and were fabricated so there was a 40-cm. length of the 31-mm. quartz tubing between joints. The entire vertical combustion assembly (dropping funnel, metering valve, quartz combustion tube, and Schwartz U-tube) was arranged to enable vertical movement such that the quartz tube could be moved up or down in the furnace as desired while maintaining the integrity of all connections. The temperature indicated by the pyrometer with its thermocouple embedded in the packing in the quartz tube of the air purification furnace was assumed to be the same as that inside the analytical quartz combustion tube when equal voltages are applied to the identical furnaces. To facilitate delivery of equal voltages to the furnaces, a plot of transformer setting *vs.* voltage was prepared.

Reduction Apparatus. An apparatus (Figure 2) which is a modification of the one described by Gustafsson (1960b) was used.

Glassware. The three bubblers used in the air purification train (Figure 1, *B*) were rinsed before use with double distilled water. Other glassware involved, from the point at which the SO_2 formed during combustion is trapped in the three glass bubblers (Figure 1, *E*) through measurement of the absorbance of the methylene blue, was washed with 6*N* HCl as suggested by Gustafsson (1960a,b). This was followed by thorough water rinsing including three final rinses with double distilled water. In addition, the glassware involved in the preparation of the reducing reagent and in the reduction apparatus was rinsed twice with purified water and dried before use. The zinc acetate trap (Figure 2, *F*) and the washing trap (Figure 2, *E*) were rinsed with oxygen-free purified water before using.

Reagents. Water. Pure water uniformly free of sulfur was used. Gustafsson (1960b) recommended the use of vacuum-boiled deionized distilled water. In this study, laboratory supplied glass-distilled water was redistilled twice in an all glass laboratory still with the first redistillation being made from potassium permanganate. During these distillations, the possibility of contamination of the distillate by absorption of sulfur compounds from the laboratory air was prevented by protecting the receiver with a U-trap containing dilute sodium hydroxide. This water, hereafter referred to as purified water, was used in all stages of the analytical procedure except those requiring oxygen-free water. In the reduction of sulfate and quantitative determination of the sulfide formed, the purified water was further treated to exclude oxygen and prevent its re-entry. This treatment consisted of boiling purified water 15 to 20 minutes while passing a vigorous stream of washed nitrogen through it. After boiling, a slow stream of washed nitrogen was kept flowing through the water. This water was similarly reboiled at two-week intervals.

Standard Sulfur Solutions for Addition to Fat. Practically any pure organic sulfur compound having the necessary solubility characteristics can be used as a standard. In this study, a primary standard was prepared by dissolving a weighed amount of freshly distilled thiophene in 2,2,4-trimethylpentane and diluting it to 100 ml. Additional standard solutions were prepared by diluting aliquots of the primary standard. In adding sulfur to a fat sample, a 1-ml. aliquot of the appropriate standard per fat sample was used throughout to keep the quantity of solvent per analysis constant. All 2,2,4-trimethylpentane was taken from a single batch of redistilled commercially available material.

Fat Sample. Fat samples were used which, when melted, gave a homogeneous, clear, essentially dry oil. In this study the samples were taken from a bulk sample prepared from the fatty tissue of four uncooked fowl by the pressing, centrifuging, washing, and filtering steps described elsewhere (Pippen *et al.*, 1969).

Catalyst and Support. Alcoa catalytic alumina tabular grade T-71, 1/4-inch to 8 mesh was used for the support. Vanadium pentoxide (K and K Laboratories, 99.9%) was converted to vanadyl oxalate and deposited on the catalyst support as described by Wilson and Straw (1950). Once ignited, as described below, the catalyst lasted indefinitely, but it was occasionally renewed when replacing a broken combustion tube.

Hydrogen Peroxide. The 10% peroxide solution used in the three air scrubbers (Figure 1, *B*) and the 3% peroxide used in the three sulfur dioxide absorbers (Figure 1, *E*) were prepared just before each combustion by diluting 30% hydrogen peroxide with purified water. Select 30% hydrogen peroxide containing (according to the manufacturer's label) a maximum of only 2.5×10^{-4} to 3.0×10^{-4}% sulfate (instead of the usual 5×10^{-4}%) to prepare the 3% peroxide solutions used in the analytical sulfur dioxide absorbers.

Paraffin Oil. A bulk supply of commercially available white, light, N.F. paraffin oil was prepared by washing it first with five successive 100-ml. portions of 2*N* KOH and then exhaustively with purified water. The washed oil was cen-

trifuged and the bulk of the clear oil decanted. A 1-ml. portion of this oil was used, for each fat sample analyzed to rinse the final amount of fat from the dropping funnel into the furnace. However, very little if any of this rinsing oil was permitted to enter the furnace.

SODIUM HYDROXIDE. A 0.1*N* solution was prepared with purified water.

SULFATE REDUCING SOLUTION. In a 1-liter spherical flask, 10.00 grams of $NaH_2PO_4 \cdot H_2O$ was dissolved in a mixture of 100 ml. of glacial acetic acid and 400 ml. of hydriodic acid (sp. gr. 1.7 and taken from a newly opened flask). After fitting the flask with a condenser and a gas delivery tube, each fitted to the flask with ground glass joints (no stoppers or corks), the mixture was boiled under reflux for 1 hour while passing washed nitrogen through it at about 50 to 100 ml. per minute. After cooling in a stream of nitrogen, the flask was closed with a glass stopper and stored in subdued light. [Caution: Fuming acid is formed upon passing gas through at room temperature (Steyermark, 1951).]

HYDROGEN SULFIDE ABSORBING SOLUTION. Zinc acetate (0.25 mole) and sodium acetate (0.10 mole) were dissolved in oxygen-free purified water and diluted to 1 liter. Traces of heavy metals were removed as described by Gustafsson (1960a).

DIAMINE SOLUTION. *N,N*-dimethyl-*p*-phenylenediamine sulfate (Eastman No. 1333), 0.93 gram, was dissolved in 750 ml. of oxygen-free purified water. Concentrated H_2SO_4 (3.50 moles) was added and, after cooling, was diluted to 1 liter with oxygen-free purified water.

FERRIC SOLUTION. Ferric ammonium sulfate [$NH_4Fe(SO_4)_2$, 0.25 mole] and concentrated H_2SO_4 (0.5 mole) were dissolved in oxygen-free purified water, cooled, and diluted to 1 liter.

NITROGEN. High purity commercially available nitrogen used in the preparation of the reducing reagent, in the reducing apparatus, and in the preparation of oxygen-free water was first passed through a trap containing 2% potassium permanganate saturated with mercuric chloride (Figure 2, *A*) and then through a trap containing purified water (Figure 2, *B*).

SULFUR-FREE SILICONE LUBRICANT. Prepare as described by Johnson and Nishita (1952).

Procedure. REDUCTION OF SULFATE AND PREPARATION OF STANDARD CURVE. A plot of sulfur *vs.* absorbance was prepared by reducing known amounts of K_2SO_4 to hydrogen sulfide and determining the absorbance of the methylene blue color developed. This plot should result in a straight line passing nearly through the origin. To carry out the reduction, 1 ml. of an aqueous solution containing a known amount of K_2SO_4 and 1 ml. of 0.1*N* NaOH were placed in the reduction flask (Figure 2, *C*). The reduction flask, with a cap clamped to the side arm and a beaker in place over the 28/5 socket joint, was then placed in an oven at 130° C. for 2 to 3 hours to permit the sample to evaporate to dryness. The reduction flask was fastened to the reduction apparatus and reduction, H_2S trapping, and development of methylene blue were carried out exactly as described by Gustafsson (1960a,b), except 5 ml. instead of 3 ml. of the reducing reagent was used and the color was developed at an ambient temperature of 25° C. instead of 20° C. Absorbance of the methylene blue was determined in 1-cm. cells on a Beckman DU spectrophotometer at 667 mμ, 0.05 mm. slit width, and with the red-sensitive phototube. Purified water was used in the reference cell since there was no difference between its absorbance and that of the blank color forming reagents.

PACKING AND IGNITION OF QUARTZ COMBUSTION TUBES. The bottom of the combustion tube was packed to a depth of 6 to 7 cm. with coarse quartz chips, a 20- to 23-cm layer of the catalyst-coated alumina granules was added and this was topped off with a 4- to 5-cm. layer of coarse quartz chips. The quartz tube in the air purification train was similarly packed, but in addition a thermocouple was included. The thermocouple leads and junction were sheathed and enclosed in small diameter quartz tubing to provide electrical insulation and protection from the catalyst and hot air. The thermocouple was inserted from the top when about half the catalyst-coated alumina had been added and, while keeping it positioned along the axis, packing of the annular space around the thermocouple and its leads was continued. The thermocouple leads were threaded through a glass head equipped with a 35/25 ball joint and a side arm for admitting air, the head was clamped in place, and the thermocouple lead taped to the glass head with thermal-setting glass tape. The packed quartz tubes were inserted in their respective furnaces and, using ball and socket joints and glass and tygon tubing, the lower end of the quartz tube in the air purification furnace was connected to the inlet end of the three bubblers in the air purification train each having been charged with 25 ml. of 10% H_2O_2. Ice was packed around these three bubblers and their air outlet was connected to the air intake on the combustion head clamped to the top of the analytical quartz combustion tube. The capillary sample inlet on the combustion head was capped off and, by applying vacuum at the Schwartz U-tube, air was drawn through the system at about 1 liter per minute while gradually raising the temperature in each furnace to 850° C. These conditions were maintained for 12 hours during which the vanadyl oxalate was converted to the yellow V_2O_5.

BLANK COMBUSTIONS. These were carried out just like fat combustions except that 10 ml. of purified water was "burned" instead of fat and no paraffin oil rinse was used. Successive blank combustions were carried out following ignition of the combustion tube until the blank value reached a uniformly low level. Blank combustions were also performed before each fat analysis to determine whether the apparatus was functioning properly and also to furnish the blank value for the subsequent analysis.

FAT COMBUSTION. While the furnaces were being brought up to 1000° C. (in interim periods they were maintained at about 200° C.) 5 to 10 ml. of the melted fat was weighed into the dropping funnel, which was loosely stoppered and clamped in place to and above the metering valve where heat from the furnace kept it in the liquid state. Into each of the three samples absorbers (Figure 1, *E*) 25 ml. of purified water and 3.0 ml. of 30% H_2O_2 were added and into each of the three absorbers in the air purification train (Figure 1, *B*) 25 ml. of 10% H_2O_2 was added. The inlets and outlets of these absorbers were capped off and the absorbers were surrounded with ice from this point until combustion was completed. When the furnaces have reached operating temperature (*ca.* 1000° C.), the air purification train was attached and air was drawn through it into the top and out the bottom of the combustion tube at about 2 to 3 liters per minute for about 10 to 15 minutes by applying vacuum directly to the Schwartz U-tube (thus bypassing the sample absorbers). Then the air flow was interrupted, the sample absorbers were connected, and air was drawn through the system at a 7 liters per minute rate. With the metering valve closed, the stopcock in the dropping funnel was opened and then the metering valve was gradually opened while watching for the appearance

of fat in the capillary delivery tube. The rate of fat addition was adjusted so that it was added no faster than 1 drop every 5 seconds, and the flow rate carefully monitored throughout the combustion. At a flow rate of 1 drop per 5 seconds, a 10-gram sample of oil can be cleanly burned in 1 hour. When lesser amounts of fat were burned, we used a slower rate of fat addition to keep the total combustion time at about 1 hour. When nearly all the fat has been added and the last bit has drained into the constriction in the dropping funnel just above the stopcock, the funnel was rinsed with 1 ml. of paraffin oil delivered from a pipet. The addition was carefully monitored at this point and when it became evident that all the fat had been added and that paraffin oil was about to break through, the metering valve was closed. The combustion tube was lowered until the bead on top of the 35/25 quartz socket joint was about 6.5 cm. above the top of the furnace. A split-sleeve insulator (surrounding the combustion tube just above the furnace) of such length that the lower portion of the socket joint just touches the insulator when the combustion tube is lowered the proper distance facilitates this operation. At this point, without changing the power input to the furnace, an air-flow rate of 7 liters per minute was maintained for 10 minutes; then 4 liters per minute for 5 minutes; then 1 liter per minute for 5 minutes; and finally about 0.5 liter per minute for 10 minutes or until, by visual observation down into the combustion tube, no unburned carbon was evident. Combustion is now complete.

The air-flow rate was reduced to a very low level. The sample absorbers were first disconnected from the combustion tube (to prevent any possibility of suck-back) and then the vacuum at the other end of the sample absorption train was promptly disconnected. Power to the furnaces was reduced to stand-by level, the combustion tube raised to its starting position, the dropping funnel removed, and the metering valve and combustion head removed as a unit to prevent oil held up in this assembly from entering the furnace. The combustion tube is now ready for the next combustion; both it and the combustion tube in the air purification train were capped off between analyses to prevent contamination.

Preparing Sulfate from Combustion for Reduction and Determination as Hydrogen Sulfide. Following combustion, contents of the three sample absorbers were quantitatively transferred with purified water rinses, to a 500-ml. spherical flask. Excess hydrogen peroxide was destroyed by placing a platinized platinum grid (cylindrical shaped approximately 5 cm. long by 1 cm. diameter and equipped with a platinum handle) in the flask, attaching a standard length 24/40 joint to act as a partial reflux condenser, and gently boiling the contents for 15 minutes using an electric heating mantle. Then, 1.0 ml. of 0.1 *N* NaOH was added and boiling continued an additional 15 minutes. After cooling, the volume was reduced to 3 to 5 ml. on a rotary vacuum concentrator and the concentrate was transferred with purified water rinses to the reduction flask (Figure 2, *C*). A standard length 28/15 ball joint was attached to the reducing flask and purified nitrogen was passed through the solution *via* the capillary side arm while the flask was being heated with a heating mantle until the solution was concentrated to 1 to 3 ml. At this point, the capillary side arm was capped off, a small beaker placed over the 28/15 socket joint, and the sample taken to dryness by placing it in an oven at 130° C. for 2 to 3 hours. The sample is now ready for reduction and determination of the H_2S produced as described above.

Reagent Blank. Omitting the combustion step, 9.0 ml. of the low sulfate 30% hydrogen peroxide solution was pipetted into a 500-ml. spherical flask. Purified water, approximately equal to the sum of that quantity of water in the absorbers, after a combustion and that quantity used to rinse out the absorbers, was added and destruction of peroxide, evaporation to dryness, etc., was carried out as described above.

Table I. Comparison of Reagent Blank and Combustion Blank

	Reagent Blank	Combustion Blank
Number of determinations	15	65
Mean absorbance[a]	0.035	0.043
Mean sulfur value, μg.	2.5	3.3
Range of sulfur value, μg.	1.5–3.3	1.6–5.3
Standard deviation (σ), μg. sulfur	0.3	0.5
Mean sulfur value $\pm 2\sigma$, μg. sulfur	1.9–3.1	2.3–4.3

[a] *t*-Test of absorbance values of reagent blank and combustion blank shows they are significantly different ($t = 3.098$; $t_{0.01} = 2.640$).

Calculation of Quantity of Sulfur in Fat. The net absorbance was determined by subtracting the combustion blank absorbance from sample absorbance. Using the net absorbance, the total number of micrograms of sulfur in the sample was determined from the standard curve. Dividing this value by the weight of the fat sample gives micrograms of sulfur per gram of fat.

RESULTS AND DISCUSSION

The combustion blank was significantly greater than the reagent blank (see footnote comparing mean absorbancies, Table I). Therefore it is essential that the combustion blank, and not the reagent blank, be used as the analytical blank.

Most of the sulfur in the combustion blank (2.5 out of 3.3 μg. or about 76%) comes from the reagents (see mean sulfur values, Table I). Thus, hydrogen peroxide, water, sodium hydroxide, and reagents used in the reducing procedure are all possible sources of the sulfur in the reagent blank. We were unable to detect any sulfur in our water. Furthermore, reagent grade sodium hydroxide typically contains only 0.001% sulfate; hence, the 1 ml. of 0.1*N* sodium hydroxide used per sample would contribute only about 0.01 μg. of sulfur, or less than 1%, to the reagent blank. The entire reducing procedure, including development of methylene blue, evidently contributes little to the reagent blank for our standard curve passed very nearly through the origin. Indeed, Gustafsson (1960b) has shown that the blank for the reducing procedure has an absorbance of about 0.002 (1-cm. cells). An absorbance of 0.002 amounts to only about 5.7% of the reagent blank absorbance (0.035, Table I). Hence the reducing procedure itself contributes little to the reagent blank. The 9 ml. of 30% hydrogen peroxide used in each analysis is therefore the main source of sulfur in the reagent blank and the reagent blank is thus essentially an analysis for sulfate in the hydrogen peroxide solution. The desirability of using hydrogen peroxide solution that is low in sulfate is thus apparent. Although we did not try it, it would seem feasible to reduce substantially the reagent blank, and hence the combustion blank, by removing the sulfate from the peroxide solution with an ion exchange resin.

The small amount of sulfur (it averages 0.8 μg., Table I) that comes from the furnace during a blank combustion is somewhat variable and thus explains why the combustion blank varies considerably more than the reagent blank (compare ranges and standard deviations, Table I). Apparently,

the catalyst and catalyst support absorb and desorb small amounts of sulfur somewhat unpredictably. If the quantity of this sulfur could be substantially reduced or made more constant the precision of the method could be considerably improved. We did not study any variables that might accomplish this. But it does appear that a less absorptive catalyst and catalyst support would help and that lengthening the after-burning period following completion of combustion might also be effective.

The addition of 1 ml. of 2,2,4-trimethylpentane (TMP), the quantity used to dissolve and add known amounts of sulfur to fat samples, did not significantly affect the sulfur content of the fat (compare I and II and also see *t*-Test in footnote, Table II). Therefore the TMP solvent itself did not contribute detectable sulfur to the fat. Consequently, the very highly significant difference between the sulfur content of groups II and III (Table II) is due only to the addition of 5 μg. of sulfur to each of the samples in group III. Thus, by analyzing 10-gram samples, the method is easily capable of distinguishing between fat samples which differ by only 0.5 μg. of sulfur per gram of fat. This discriminatory level was low enough for the authors' requirements, but it should be possible by burning 20-gram samples to distinguish readily between samples which differ by only 0.25 μg. of sulfur per gram of fat. Still lower discriminatory levels would seem practically attainable by increasing the cell path length in the spectrophotometer, by collecting the H_2S in a smaller volume, and by developing ways to reduce the variation in the combustion blank.

Although recovery of 5 to 20 μg. of sulfur added to 10 grams of fat is considerably less than 100% (Table III), the actual recoveries (73 to 90%, Table III) are remarkably good when it is recalled that ultra micro amounts of sulfur are being recovered from macro-sized samples in a rather complex series of analytical steps.

Table II. Sulfur Content of a Fat Sample as Influenced by Addition of Solvent and Sulfur

Group	Added to Each 10-Gram Sample	Replicate Analyses	Mean Sulfur Value,[a] μg./g. fat	Standard Deviation, μg./g. fat
I	Nothing	8	0.738	0.077
II	One ml. 2,2,4-trimethylpentane	8	0.697	0.113
III	One ml. 2,2,4-trimethylpentane containing 5 μg. sulfur	8	1.061	0.112

[a] *t*-Test for differentiation between mean sulfur values: I *vs.* II: The difference is not significant (t = 0.843; $t_{0.05}$ = 2.145). II *vs.* III: The difference is significant (t = 6.582; $t_{0.001}$ = 4.140).

Table III. Recovery of Known Amounts of Sulfur Added to Approximately 10-Gram Samples of Fat Taken from a Bulk Fat Sample

Number of Analyses	Sulfur: Added per Sample, μg.	Sulfur: Mean Recovery per Sample, μg.	Mean Recovery, %
8	5	3.64	72.8
2	10	9.03	90.3
2	20	17.10	85.5

LITERATURE CITED

Bertolacini, R. J., Barney, J. E., II., *Anal. Chem.* **29,** 281 (1957).
Fildes, J. E., Kirsten, W. J., *Microchem. J.* **9,** 411 (1965).
Franks, M. C., Gilpin, R. L., *J. Inst. Petrol.* **48,** 226 (1962).
Gustafsson, L., *Talanta* **4,** 227 (1960a).
Gustafsson, L., *Talanta* **4,** 236 (1960b).
Heinrich, B. J., Grimes, M. D., Puckett, J. E., "Treatise on Analytical Chemistry," Analytical Chemistry of the Elements, Part II, Vol. 7, pp. 1–135, Interscience, New York, 1961.
Hudy, J. A., Dunn, C. L., J. AGR. FOOD CHEM. **5,** 351 (1957).
Hudy, J. A., Mair, R. D., *Anal. Chem.* **27,** 802 (1955).
Johnson, C. M., Nishita, H., *Anal. Chem.* **24,** 736 (1952).
Lambert, J. L., Yasuda, S. K., Grotheer, M. P., *Anal. Chem.* **27,** 800 (1955).
Lloyd, A. G., *Biochem. J.* **72,** 133 (1959).
Lysyj, I., Zarembo, J. E., *Microchem. J.* **3,** 173 (1959).
Milner, O. I., "Analysis of Petroleum for Trace Elements," pp. 87–95, Macmillan, New York, 1963.
Pippen, E. L., Mecchi, E. P., Nonaka, M., *J. Food Sci.* **34,** 436 Sept.–Oct. (1969).
Polsky, J. W., Betz, W. H., Betz, L. D., *Anal. Chem.* **19,** 657 (1947).
Spencer, B., *Biochem. J.* **75,** 435 (1960).
Steyermark, A., "Quantitative Organic Microanalysis," p. 235, Blakiston, Philadelphia, 1951.
Stoffyn, P., Keane, W., *Anal. Chem.* **36,** 397 (1964).
Wilson, H. N., Straw, H. T., *J. Soc. Chem. Ind.* (London) **69,** 79 (1950).

Received for review June 16, 1969. Accepted December 15, 1969. Reference to a company or product name does not imply approval or recommendation of the product by the U. S. Department of Agriculture to the exclusion of others that may be suitable.