

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE SOUTH CAROLINA FOOD RESEARCH COMMISSION, AND THE LABORATORY OF PHYSIOLOGICAL CHEMISTRY, UNIVERSITY OF MINNESOTA MEDICAL SCHOOL]

THE DETERMINATION OF TRACES OF IODINE. III. IODINE IN MILK, BUTTER, OIL AND URINE¹

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In the first paper of this series² was described a combustion tube method for the destruction of organic matter in foodstuffs for the purpose of determining traces of iodine. This method has since been used by Reith,³ Schwaibold,⁴ and others, and is the only workable method which we have found for iodine estimation in some classes of organic material. The second paper⁵ describes a method of introducing dried vegetables into the tube, and some improvements which experience has suggested in the manipulation. It also describes a method of low temperature ignition in a muffle which has been found reliable for root and leafy vegetables (with a loss of about 15%) and which materially simplifies and shortens the technique. This alternative method was checked on known quantities of iodine added to potatoes, and on dried thyroid added to potatoes, with very satisfactory results.⁶ When applied to skim milk powder this method sometimes failed. The loss was evidently sometimes due to too high a temperature in the first heating or smoldering of the sample. Duplicate samples done by the same chemist would check very accurately. Sometimes another pair of duplicates of the same sample done by another chemist would check but not check with the first pair. The smoldering must be carefully watched so that no live coals appear at the surface, in order to prevent loss. This is especially true of evaporated urine residues, particularly if only calcium oxide is added as an alkali. In this case only about 80% of added iodide was recovered. The low temperature method depends on the presence of a great excess of alkali and is, therefore, best adapted to vegetables other than cereal grains. Loss of iodine in ashing other samples can be prevented only by large additions of alkali and, therefore, to a certain extent defeats the purpose of the method, which is the freeing of the iodide from any large amount of other substances. The

¹ Aided by a grant from the Joint Committee, University of Minnesota and Mayo Foundation, and from the Committee on Therapeutic Research, Council on Pharmacy and Chemistry, American Medical Association.

² J. F. McClendon, *THIS JOURNAL*, **50**, 1093 (1928).

³ J. F. Reith, "De Micro-jodiumbepaling in Natuurlijke Grondstoffen," Dissertation, Utrecht, 1929.

⁴ S. Schwaibold, "New Method for Determining Iodine in Organic Substances," *Chem.-Ztg.*, **54**, 22 (1929).

⁵ J. F. McClendon and Roe E. Remington, *THIS JOURNAL*, **51**, 394 (1929).

⁶ R. E. Remington, F. B. Culp and H. von Kolnitz, *ibid.*, **51**, 2942 (1929).

low temperature ashing method is not applicable to whole milk, fatty seeds, nuts or fats and oils. The present contribution will, therefore, describe methods which have been found suitable for these products, and some additional improvements in the original procedure.

Method for Fat (Including Butterfat).—The fat is melted and, if necessary, filtered free from water and suspended impurities. It is then introduced into the bottle of a large atomizer or lacquer spray apparatus (New Way Spray Gun Co., Cleveland), and sprayed into a silica tube (1 inch bore, 2 feet long with elbow 1 foot long) by means of a current of oxygen. The general set-up (Fig. 1) is the same as described in the preceding paper with some exceptions. It is necessary that the silica tube be very hot

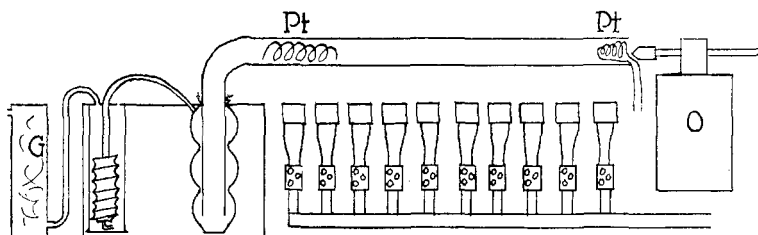


Fig. 1.—Combustion apparatus for burning fat and oil. The oil is placed in the container (O) of the atomizer and sprayed with oxygen into the silica tube, 2 feet long, 1 inch bore, with elbow 1 foot long. A platinum spiral (Pt) right in front of the atomizer tip is kept at white heat by means of an electric current. Another platinum spiral (Pt) further in the tube is heated by means of Meker burners under the tube. The elbow in the silica tube dips into an absorption flask of three compartments and the joint is packed with asbestos fiber. This is connected with ten Milligan wash-bottles in parallel, all immersed in ice water, which are connected with a tower packed with glass wool (G) and finally with an exhaust pump.

during the combustion or that a point in front of the atomizer be kept hot by means of a minute blast-flame or electrically heated platinum spiral. If electric heat is used outside the tube some provision for visibility should be made. A piece of platinum foil⁷ or a platinum spiral of heavy wire in the tube acts as a catalyst. To close the end of the tube and prevent carbonization at the nozzle, it is surrounded with a pad of moist asbestos, which can be cooled by dropping water on it. The sodium hydroxide in the first wash bottle or absorption flask has been replaced by 0.25 g. of sodium sulfite, which insures that the iodine is reduced to the iodide form. In order to hasten the combustion, a rotary air pump drawing 2 cu. ft. of air per minute is used to suck the exhaust gases through the apparatus and ten Milligan wash bottles are used in parallel. It is necessary to cut the gas stream velocity down to a point where absorption is complete. All wash bottles are immersed in ice water. Following the wash bottles one or more towers packed with glass wool or an alundum extraction thimble to filter particles from the exhaust gases is inserted.

The adjustment of the atomizer is very important, for if the ratio of oxygen to fat is too high, the fatty acids will not burn, but will distil over. If, on the other hand, too little oxygen is used, soot will form. The atomizer is best adjusted in a preliminary

⁷ A tube lined with a platinum film soon cracked. A manganous salt added to the fat as a catalyst did not improve the combustion. Probably a rare earth salt would be useful.

trial and kept set during the combustion. The amount of fat burned is determined by weighing the atomizer bottle before and after the combustion. If the iodine content is low, it may be necessary to burn as much as 500 g. of fat. It is possible to so conduct the burning that no soot and tarry products are formed. The liquid in the wash bottles is placed in a beaker, the rinsings of the apparatus added, evaporated to small volume, transferred to a nickel boat, evaporated to dryness and ignited in a pyrex tube (Fig. 2), using about 5 cc. of water and a few milligrams of sodium sulfite in a side-necked test-tube to absorb volatilized iodine. If an alundum capsule is used it is ignited in the same tube. The boat is placed in the smallest test-tube into which it may be introduced, the sulfite solution added, together with rinsings of the tube, and left until the ash has dissolved. The solution is centrifuged free from insoluble residue, decanted into a 30-cc. beaker, a few drops of sulfurous acid (to reduce iodate) and a few milligrams of sodium azide added (to decompose nitrites) and neutralized with sirupy phosphoric acid, using bromphenol blue paper as indicator. Four drops of phosphoric acid are added in excess and the solution boiled to remove sulfur dioxide (as well as excess hydrazoic acid).

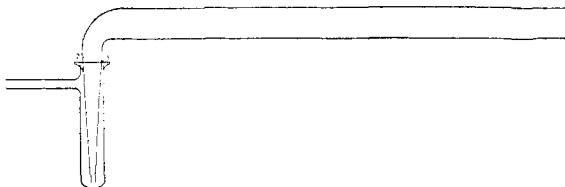


Fig. 2.—Small pyrex combustion tube and side-necked test-tube for absorbing iodine from the combustion.

If the odor of hydrazoic acid is not perceived, more sodium azide is added. It was shown by Reith that there might be considerable nitrite formed in the combustion of foodstuffs containing nitrogen, and that the nitrite might be decomposed by hydrazoic acid.

The solution is now transferred to a small separatory funnel with an etched mark at 10 cc., made up to the mark with distilled water and about 1 mg. of sodium nitrite added, followed by 1 cc. of purified carbon tetrachloride. The separatory funnel is shaken for at least two minutes, during which time the iodide is oxidized to iodine and 85/95 of the iodine passes into the carbon tetrachloride solution and is compared with a standard containing 0.1 mg. of iodine per cc. in a Bausch and Lomb micro-colorimeter. This is multiplied by 1.118 to correct for the partition coefficient of iodine between carbon tetrachloride and the dilute salt solution. Or, since this partition coefficient varies

TABLE I
IODINE CONTENT OF BUTTERFAT

Town	County	State	Iodine, parts per billion
.....	Minnesota	4.0
St. Paul	Ramsey	Minnesota	11.0
Orono	Maine	13.0
Hasson	Minnesota	14.3
.....	Minnesota	15.7
Nelson and Wadena	Minnesota	16.5
Davis	California	26.3
Wadena	Minnesota	28.9
Charleston	South Carolina	52.7
Clemson College	South Carolina	78.0

slightly with variation of salt content, the solution may be repeatedly extracted with carbon tetrachloride and the yields of the different extractions added together.

By this method the iodine content of a sample of Norwegian cod-liver oil was found to be 5800 parts per billion.

Method for Oily Seeds and Nuts and Milk.—On account of the large amount of fat or protein present, low-temperature ignition is not successful on this class of products, even though one is content with 85% recovery, and since the material cannot be sprayed, the tube furnace with the screw feed is used. Visking sausage casing has been found very satisfactory as a lining in which to pack the dry material before introduction into the screw feed device. The mouth of the feed tube ($\frac{7}{8}$ -inch bore) is brought into juxtaposition with the end of the silica tube furnace (1-inch bore) and oxygen delivered to the crack by a nozzle made from an ordinary flame spreader ("fish-tail" or "wing-top" for Bunsen burner) which has been bent into a curve to fit the outside of the feed tube (Fig. 3, the detail above the diagram shows the flame spreader from another angle).

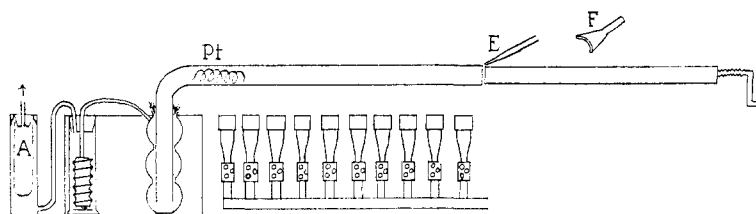


Fig. 3.—Combustion apparatus for burning solid food materials. The arrangement is the same as in Fig. 1, except that the atomizer is replaced by a steel tube of $\frac{7}{8}$ -inch bore with a micrometer screw for forcing the foodstuff into the silica tube. The foodstuff is packed in a Visking sausage casing which is inserted in the steel tube. Oxygen is emitted through the adapter (E) which is shown at perspective at (F). The tower of glass wool shown in Fig. 1 is replaced by an alundum capsule (A) for straining out the particles of smoke that might escape the absorption flask.

About 6 inches of the silica tube is lined with sheet iron or nickel for protection from the ash. After igniting the material by means of a flame, the joint is covered with a lump of wet asbestos fiber and kept moist. The combustion is then carried on as previously described. The ash is ground for one hour with 100 cc. of 95% alcohol in a 250-cc. ball mill and then for one hour with 100 cc. of 80% alcohol; the combined extracts are centrifuged and the combined alcohol extracts decanted, evaporated and added to the evaporated washings of the apparatus. If a yellow or dark color appears, the residue must be transferred to a nickel boat and ignited in the small tube, Fig. 2.

Milk is best neutralized with sodium hydroxide, using phenol red paper as indicator, evaporated to small volume on a water-bath, some rare earth (ceria or thoria) and sulfur added, heated in an oven at 100° until dry, then ground in a burr mill or broken into pieces and packed in Visking sausage casing. The evaporation of milk in open dishes is tedious and wherever possible milk powder is obtained. The rare earth seems to act as a catalyst in combustion,⁸ and the sulfur aids in the reduction of iodine.

Geographical surveys on the iodine content of milk are meaningless unless considerable data are collected, since it has been shown that the feeding of iodine compounds increases the iodine content of the milk, and there are now on the market many dairy feeds which contain fish

⁸ Personal communication of O. S. Rask.

meal, ground kelp and mineral iodides. The iodine content of whole milk may be calculated from the iodine content of butterfat and skim milk. We were able to determine the iodine content of the "hulls" of the fat droplets after feeding a cow iodide, but ordinarily we think this fraction is negligible.

Since butterfat is used for human consumption to a greater extent than are the other constituents of milk, it is of interest to know the ratio of the iodine in the butterfat to that in the skim milk of the same sample of milk. For this purpose about five gallons of milk are separated, the cream is churned and the total iodine content of the skim milk plus butter-milk determined separately from that of the butter.

TABLE II
DISTRIBUTION OF IODINE IN COW'S MILK

Town	County	State	Feed	Iodine in parts per billion		Ratio of total iodine in butter to that in skim milk
				Butterfat	Dry skim milk	
Clemson College	S. C.	Normal	78	142	0.9
Davis	Cal.	Normal	26	164	.04
Byron Rock						
River Farm	Ill.	Kelp and fish	176	400	.7
St. Paul ^a	Ramsey	Minn.	Normal	11
St. Paul	Ramsey	Minn.	Cod-liver oil	36	7,320	.002
St. Paul	Ramsey	Minn.	Iodized oil, ^b 100 g. daily	43	65,600 ^c	.0006
St. Paul	Ramsey	Minn.	Iodized salt containing 10% iodine	378	158,000	.0004
St. Paul	Ramsey	Minn.	4-6 Days after discontinuing iodized salt	148	2,800	.02

^a With coöperation of Dairy Division, Department of Agriculture, University of Minnesota.

^b Made by adding 20 g. of ICl_3 to a gallon of linseed oil.

^c Iodine in hulls, 26,000.

The above results of feeding a cow iodized salts equivalent to 10 g. of iodine a day for five days and another cow iodized oil are not in agreement with those of Scharrer and Schwaibold⁹ or of Rasche.¹⁰ It seems probable that these discrepancies are due to losses of iodine in analysis and we doubt whether accurate analyses can be made on samples much smaller than ours by any method.

Method for Urine.—If the urea is not destroyed it becomes troublesome to burn the urine residue because the decomposing urea forms gases which foam and sputter, causing loss and putting out the flame. The twenty-four hour urine specimen is colored with phenol red, neutralized

⁹ K. Scharrer and J. Schwaibold, "Über die Art der Bindung des Jods in der Milch," *Biochem. Z.*, **207**, 333 (1929).

¹⁰ W. Rasche, *Z. Kinderheilkunde*, **42**, 124 (1926).

with lime water and subjected to hydrolysis with urease (about 5 cc. of the extract of 5 g. of jack bean meal). An hour suffices for the hydrolysis of the urea, after which 5 g. of lime is added and the urine evaporated to a small volume. (The use of a hot-plate regulated at 100°, Fig. 4, avoids the danger of overheating.) When evaporated to small volume the urine is transferred to a nickel boat, some rare earth and sulfur added, evaporated to dryness and ignited in an excess of oxygen in a pyrex tube furnace (Fig. 5) by first heating the material nearest the absorption flask by means

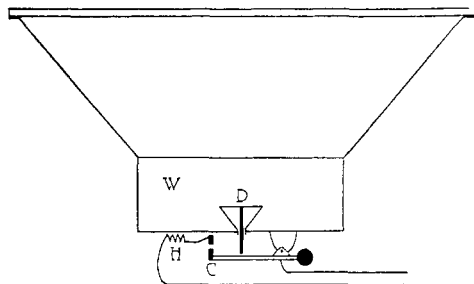


Fig. 4.—Constant temperature hot-plate, kept at 110° by means of an electric regulating apparatus. The hot-plate is the top of an evacuated chamber, the lower portion of which is filled with water (W) and heated by the electric heater (H). When the water (W) boils and produces steam above atmospheric pressure, the diaphragm (D) is pushed down, forcing down the electric contact (C) and thus breaking the circuit and cutting off the heat.

of an oxy-gas torch or electrically heated platinum loop and working back to the open end of the tube.

The pyrex tube furnace shown in Fig. 5 is reduced in size stepwise so as to accommodate nickel boats of different sizes. The platinum spiral is heated by the electric current from a transformer in which the voltage may be varied by turning a knob (Franklin Transformer Company, Minneapolis). It may be heated by means of a gas flame below the tube, but there is more danger of softening the pyrex. The spiral is heated so as to be luminous in a darkened room but not perceptibly luminous in a brightly lighted room. The oxy-gas torch has an opening made by driving a needle through the oxygen tube, thus directing a stream of oxygen forward so as to supply oxygen to the platinum spiral and complete the combustion of gases. The torch itself has the gas cut down to a small rate and the oxygen supplied in excess so that the heated urine residue will burn with a dazzling luminosity. In case an electrically heated platinum loop is substituted, an attached tube carries oxygen, which is directed toward the loop. The loop is plunged into the urine residue and moved slowly backward until all is ignited.

If any tarry film condenses on the pyrex tube it is burned off by placing a gas flame under it.

At the end of the combustion the ash has a light gray color or is almost white. The ash is ground for one hour in a 250-cc. ball mill with 100 cc. of 95% alcohol, followed by one hour with 100 cc. of 80% alcohol, and the combined extracts are centrifuged and decanted. The extracts, sul-

fite solution and washings of the tube are evaporated in a porcelain dish to a small volume and, if necessary, centrifuged again and transferred to a nickel boat, evaporated to dryness, and ignited in the smaller pyrex tube (Fig. 2). The analysis is then continued as in analysis of iodine in fat.

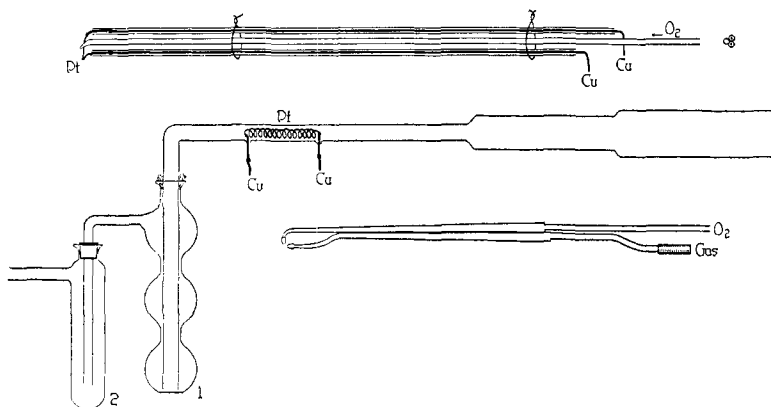


Fig. 5.—Apparatus for burning urine residue. The urine is placed in a nickel boat and is inserted into the pyrex tube which is narrowed step-wise. The boat is placed in the narrowest portion which will conveniently receive it. The platinum spiral (Pt) is heated by means of the copper connection (Cu) in the middle of the figure. The pyrex combustion tube is bent down into a tripartite absorption flask (1) and the joint closed with asbestos. The second absorption flask (2) is closed by means of a rubber stopper. Heat is supplied by means of the oxy-gas torch, shown below, with an extra opening in the oxygen tube, allowing oxygen to pass forward, or by means of the apparatus shown above. To show the details the binding wires are loosened so that the three parts are shown separately. The middle tube (O) carries oxygen, which is directed toward the platinum loop (Pt) and in each of the other tubes is a pyrex tube containing a copper wire, which is welded onto a large platinum wire, which, in turn, is connected onto the platinum loop of finer platinum wire (Pt), which is heated to a white heat by means of an electric current. The gas or electric torch is held in the hand and inserted inside the pyrex combustion tube in order to burn the urine residue.

In order to determine the behavior of the apparatus, relatively large quantities of iodide were added to small samples of iodine-free substances and burned in the tube. Ten mg. of iodine (as potassium iodide solution) was added to 50 cc. of iodine-free urine treated as above described, and burned in the tube. There was recovered

8.10 mg. in the ash in the boat
 0.49 mg. in the washings of the tube
 0.47 mg. in the 1st absorption tube
 0.055 mg. in the 2nd absorption tube

Total, 9.115 mg. of iodine recovered

On repeating this experiment the following values were obtained

3.07 mg. in the ash in the boat
0.375 mg. in the washings of the tube
5.562 mg. in the 1st absorption tube
0.762 mg. in the 2nd absorption tube

Total, 9.769 mg. of iodine recovered

Ten mg. of iodine (as potassium iodide solution) was added to 5 g. of iodine-free starch and burned in the tube with the following recovery

7.94 mg. in ash in the boat
1.26 mg. in 1st absorption flask (none found in 2nd absorption flask)
0.13 mg. in washings of tube

Total, 9.33 mg. of iodine recovered

This experiment was repeated, with the following results

9.66 mg. in the ash in the boat
0.52 mg. in the 1st and 2nd absorption flasks
0.05 mg. in the washings of the tube

Total, 10.23 mg. of iodine recovered

The recovery was within the limits of accuracy of the pipet in which the iodide solution was measured. The variations in the distribution of iodine in different parts of the apparatus were probably due to variations in temperature, duration of heating and rate of suction of air and exhaust gases through the apparatus. If potassium iodide is heated in a test-tube over a Bunsen burner, elemental iodine is evolved. It is not necessary to raise the temperature sufficiently to sublime the iodide in order to carry over iodine into the absorption flasks. If there is not sufficient sulfite in the absorption flasks, iodine will color the solutions in them (and some be lost, passing into the suction pump), and the color disappears on adding more sulfite.

Eight (24-hour) urine specimens of Minnesota men who used ordinary non-iodized salt ranged between 0.008 and 0.011 mg. (av. 0.00956) of iodine. Nine (24-hour) urine specimens of Minnesota men who used iodized salt ranged from 0.068 to 0.295 (av. 0.1213 mg.). The great variability is probably due to variability of intake of iodine. The average iodized salt contains about 1.4 mg. of iodine per 100 g. of salt. The amount used by the subjects was not controlled. In two determinations on days following strenuous exercise, the iodine excreted in the urine was double that on other days, but the iodine intake was not investigated. In one determination day-urine contained about twice as much iodine as night-urine (12-hour day urine, 0.09, and 12-hour night urine, 0.05 mg. of iodine).

RESULTS OF ANALYSES

Subject	Mg. of iodine in 24-hour urine No iodized salt used	Subject	Mg. of iodine in 24-hour urine Iodized salt used
1	0.008	1	0.068
2	.008	2	.082
3	.009	3	.085
4	.0095	1	.102
5	.01	4	.112
5	.01	4	.128
2	.011	1	.128
6	.011	4	.182
		1	.205 ^a
Average	0.00956	Average	0.1214

^a Day following heavy exercise.

Summary

1. Foodstuffs and urine are burned with oxygen in a silica or pyrex tube furnace, using an atomizer for oils and a screw feed device for solid substances. Heat is applied from the outside by gas burners or from the inside by electrically heated platinum wires or oxy-gas torches. Urine is freed from urea with urease and burned in a pyrex tube furnace.

2. Sodium sulfite solution is used to absorb the iodine, and hydrazoic acid to free it from excess nitrite. The iodide is oxidized with a known quantity of nitrite to iodine and shaken out with carbon tetrachloride.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF CALIFORNIA]

**THE HEAT CAPACITY AND ENTROPY OF POTASSIUM
PERCHLORATE FROM 12 TO 298° ABSOLUTE. THE HEAT OF
SOLUTION OF POTASSIUM PERCHLORATE. THE ENTROPY
AND FREE ENERGY OF PERCHLORATE ION**

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This work is a continuation of the program^{1a} for the evaluation of entropies of aqueous ions and constitutes an example of the application of these values to the determination of the free energy of a negative ion which is not readily obtained by the ordinary equilibrium or electromotive-force methods.

The following measurements were made: (1) the heat capacity of po-

¹ Du Pont Fellow in Chemistry.

^{1a} For references to the general theory see (a) Latimer and Buffington, *THIS JOURNAL*, **49**, 2297 (1927); (b) Latimer, *ibid.*, **48**, 2868 (1926); (c) Latimer and Greensfelder, *ibid.*, **50**, 2202 (1928); (d) Latimer and Kasper, *ibid.*, **51**, 2293 (1929); (e) Latimer, *J. Phys. Chem.*, **31**, 1267 (1927).