Table IV. Retention of Original Peroxidase Activity in Blanched, Cut Sweet Corn

Blanch Time, Min.	Absorbance Units/G.
0	20.5
1	0.780
2	0.600
4	0.495
6	0.399
8	0.287

given in Table III. The enzyme concentration found was independent of the weight of sample taken for color development; therefore, there are no heat-stable compounds in whole-kernel corn that may lead to an error in the assay.

The data in Table III also indicated the reproducibility of the assay when the enzyme content was determined for several samples taken from a batch of heated corn. The enzyme concentration values ranged from a minimum of 0.52 to a maximum of 0.78. This difference constitutes a variation of 33%. This degree of accuracy is also shown by the data in Table I. Here, the variation is approximately 20% between the maximum and minimum absorbance per concentration unit values. The data in Table I were obtained by taking aliquots of a single dilution of unheated corn. Therefore, the variation should be attributed to manipulations during the assay and not to differences in the enzyme content of heated corn. However, an enzyme assay having a variation of about 30% is believed adequate for most food analyses—particularly when small concentrations such as 1% of original enzyme content are being determined.

Application of Assay to Blanched Corn. Freshly harvested sweet corn was used in applying the enzyme assay to sweet corn blanched for various times to 8 minutes. The corn was husked, removed from the cob, washed in a rod-reel washer, and blanched in steam at 210° F. The corn was air-cooled immediately after blanching.

Appropriate weights of samples were blended for color development. The enzyme concentrations were determined and the results expressed as absorbance units per gram of corn. The results in Table IV showed that increasing blanch times gave decreasing enzyme activity.

Acknowledgment

This work has supported in part by Bankhead Jones funds assigned through the State Experiment Stations Division, Agricultural Research Service.

Literature Cited

(1) Dietrich, W. C., Lindquist, F. E., Bohart, G. S., Morris, H. J., Nutting, Marvel-Dare, *Food Research* **20**, 480 (1955).
(2) Esselen, W. B., Anderson, E. E., *Ibid.*, **21**, 322 (1956).
(3) Esselen, W. B., Pflug, I. J., *Food Technol.* **10**, 557 (1956).
(4) Farkas, D. F., Goldblith, S. A., Proctor, B. E., *Food Eng.* **28**, (No. 1), 52 (1956).
(5) Guyer, R. B., Holmquist, J. W., *Food Technol.* **8**, 547 (1954).
(6) Joslyn, M. A., *Food Inds.* **18**, (No. 8), 1204 (1946).
(7) Labbee, M. D., Esselen, W. B., *Food Technol.* **8**, 50 (1954).
(8) Nebesky, E. A., Esselen, W. B., Kaplan, A. M., Fellers, C. R., *Food Research* **15**, 114 (1950).
(9) Reddi, R. R., Esselen, W. B., Fellers, C. R., *Food Technol.* **4**, 63 (1950).
(10) Wallerstein, J. S., Alba, R. T., Hale, M. G., Levy, H., *Biochim. et Biophys. Acta* **1**, 327 (1947).

FOOD IRRADIATION

Determination of Micro Quantities of Methyl Mercaptan in Gamma-Irradiated Meat

R. A. SLIWINSKI and D. M. DOTY

American Meat Institute Foundation and Department of Biochemistry, University of Chicago, Chicago, Ill.

A sensitive method for determining micro amounts of methyl mercaptan produced in gamma-irradiated meat has been developed which is based on the reaction with N,N-dimethyl-p-phenylenediamine. A soluble red-colored complex is formed when methyl mercaptan is reacted with the amine solution. Hydrogen sulfide is removed by the formation of an insoluble complex in a trapping solution containing mercuric acetate. The odorous vapors from irradiated meat are carried with a stream of nitrogen into a trapping tube containing the mercuric acetate. The color is developed by adding a mixture of the acid amine solution and Reissner's solution. The intensity of the soluble red-colored complex developed is measured in a photoelectric spectrophotometer, at a wave length of 500 mμ. The method can be used for the quantitative estimation of 5 to 110 γ of methyl mercaptan.

PRELIMINARY STUDIES by Batzer and Doty (2) to elucidate the nature of undesirable odorous compounds developed by gamma irradiation of fresh meat indicated that, among other things, the sulfur-containing compounds—hydrogen sulfide and methyl mercaptan—developed during this process of cold sterilization. Herk and coworkers (4), using gas partition chromatography and mass spectrometry, isolated and identified several simple sulfides and disulfides from gamma-irradiated meat.

Marbach and Doty (5) described a method for determining micro quantities of hydrogen sulfide from irradiated meat. The classical reaction between N,N-dimethyl-p-phenylenediamine and hydrogen sulfide in the presence of hydrochloric acid and an oxidizing agent, which resulted in the formation of methylene blue as first proposed by Emil Fischer in 1883 (3), was the basis for their improved quantitative method. The use of a saturated cadmium hydroxide–sodium hydroxide solution at a pH of 13 was an effective trapping agent for

hydrogen sulfide from irradiated meat, and no loss of trapped sulfide was observed from the trapping mixture after 24 hours ebullition with purified nitrogen.

Almy (7), using zinc acetate as a trapping agent, states that mercaptans are not absorbed and therefore do not interfere with the methylene blue reaction. Snell and Snell (7) repeated this statement. Sands and coworkers (6) found, however, that mercaptans are absorbed by zinc acetate, and a pink color, which can be measured by the use of a green filter, is formed on the addition of the diamine reagents. Marbach and Doty (5), using their cadmium hydroxide–sodium hydroxide trap, showed that mercaptans with the diamine reagent form a red-colored complex which absorbs at a wave length of 490 mμ. By proper wave length selection, interference due to the red complex is minimized so that no significant change in the methylene blue color readings results.

The method described here for determining micro quantities of methyl mercaptan in gamma-irradiated meat is based on the formation of this red-colored complex by the reaction of methyl mercaptan with N,N-dimethyl-p-phenylenediamine, hydrochloric acid, and ferric chloride, after the mercaptan is trapped with mercuric acetate.

Reagents

Amine Solution. Dissolve 5.0 grams of N,N-dimethyl-p-phenylenediamine hydrochloride in 1 liter of concentrated hydrochloric acid. The solution should have an absorbance value of 0.04 or less at 500 mμ with a 21-mm. path length. When protected from light, the solution is stable for at least 6 months.

Reissner Solution. Dissolve 67.6 grams of ferric chloride hexahydrate in distilled water, dilute to 500 ml., and mix with 500 ml. of a nitric acid solution containing 72 ml. of boiled concentrated nitric acid (specific gravity 1.42). This solution is likewise stable.

Procedure

The procedure for determining the volatile methyl mercaptan components from meat is as follows: Spray a small amount of antifoam A into a 30 × 200 mm. heavy-walled test tube. Place in the tube 20 grams of finely ground meat and 50 ml. of distilled water. Disperse the meat thoroughly by shaking the test tube vigorously. Fit the tube with a two-hole rubber stopper; in one hole, insert a 6-mm. (outside diameter) glass tube so that the end is within a few millimeters of the bottom of the test tube; in the other hole, insert a short piece of glass tubing for a Tygon tubing connection to the trapping tube.

Place a water jacket around the Tygon tubing about 6 inches above the

test tube, to condense the water vapors being carried over with the effluent gas stream. Connect the tube to the trapping tube and to the nitrogen supply and place in a water bath at 58° C. The trapping tube is a Folin-Wu sugar tube graduated at 6 and 12.5 ml., and it contains 6 ml. of 5% mercuric acetate. Immerse the trapping tube in ice water to keep the trapping solution at 0° to 4° C. Feed the effluent gas stream from the meat slurry into the trapping solution through a 6-mm. (outside diameter) glass tube drawn into a capillary tip measuring approximately 1 mm. (outside diameter).

Allow commercial high purity, water-pumped nitrogen to bubble through the meat slurry at a rate of 0.5 cubic foot per hour. After 4 hours of ebullition, disconnect the trapping tube, break off the capillary tip, and add it to the trapping tube (to retain the mercury mercaptide precipitate on the tip). Add 1.5 ml. of the acid amine solution and 0.5 ml. of the Reissner solution by means of the special cup described by Marbach and Doty (5). Stopper, invert, and shake the tube for 10 minutes to mix the reagents. Rinse the special cup allowing the rinse water to flow into the tube. Dilute to 12.5 ml. with distilled water, shake, and allow to stand at room temperature for 30 minutes. Transfer the solution to a spectrophotometer tube (21-mm. light path) and read against a distilled water blank at 500 mμ. Deduct from the reading the absorbance of a reagent solution containing no mercaptan. Read the amount of methyl mercaptan present from a standard curve prepared by using solutions containing known amounts of methyl mercaptan (see below).

Results

Trapping Agents. Methyl mercaptan does not interfere with the hydrogen sulfide assay in so far as the red complex formed with methyl mercaptan does not absorb at the maximum absorption band (670 mμ) of methylene blue (5), but the reverse is not true. Hydrogen sulfide interferes with the methyl mercaptan assay because the methylene blue formed partially absorbs in the maximum range of the red complex (500 mμ). Means of effectively trapping and assaying for methyl mercaptan were investigated and the cadmium hydroxide–sodium hydroxide solution was considered to be an effective trapping agent for hydrogen sulfide only, and methyl mercaptan was not trapped quantitatively during the process of ebullition with nitrogen gas. A "stripping effect," as described by Sands and associates (6)—or what appeared to be a "stripping effect"—occurred when nitrogen gas was bubbled through the trapping mixture containing the cadmium mercaptide complex—i.e.,
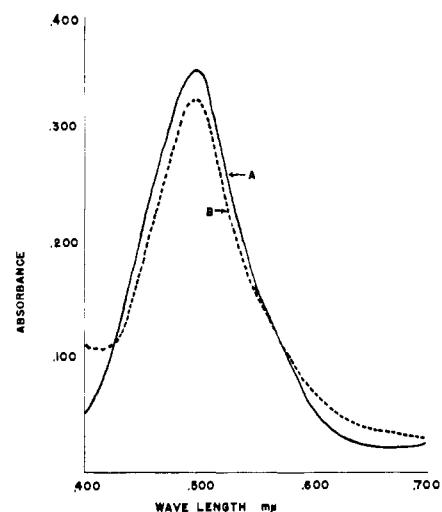

Figure 1. Absorption spectra of methyl mercaptan–N, N-dimethyl-p-phenylenediamine complex from lead methyl mercaptide (A) and from irradiated meat (B)

a loss of methyl mercaptan was noted as nitrogen was bubbled through the mixture for 4 hours (the period of time required to remove methyl mercaptan from the meat samples).

A search for a more effective trapping agent was undertaken. Various reagents were tried, but the mercury salts appeared to be the most satisfactory. Silver salts, although good trapping agents, could not be used as the amine reagent is prepared in concentrated hydrochloric acid and the formation of silver chloride would interfere with the colorimetric determinations. Mercuric acetate was chosen because of its solubility. Solutions of mercuric sulfide and of mercuric methyl mercaptide were prepared and the acid amine reactions carried out.

The absorbance of the red complex formed from methyl mercaptan, in a solution containing only mercuric methyl mercaptide, was equal to the absorbance of the red complex formed from a solution containing an equivalent amount of mercuric methyl mercaptide along with added mercuric sulfide. Only the mercury complex of methyl mercaptan reacted to form a colored complex (red). No color, whatsoever, developed with mercuric sulfide. After the color developing reactions were carried out, centrifugation of the solutions containing one or both of the sulfur-containing mercury compounds revealed that a small amount of blue precipitate was obtained only when mercuric sulfide was present. Methylene blue did not form in those tubes containing the mercury salt of hydrogen sulfide—apparently because this complex was so insoluble under the acid conditions of the reaction that no free sulfide was available for the methylene blue conversion.

Wave Length Selection. An absorption spectrum of the red complex

which forms when methyl mercaptan reacts with the acid amine reagents was plotted. Crystalline lead methyl mercaptide (see below) was accurately weighed and diluted with a 5% mercuric acetate solution. Six milliliters of this solution, equivalent to 44 γ of methyl mercaptan, was reacted with the acid amine reagents, and the absorption of the red complex was made on a Beckman Model DU spectrophotometer. The red complex absorbs maximally at 500 mμ (Figure 1, curve *A*). Curve *B* of Figure 1 shows the absorption spectrum of the red complex from irradiated meat.

Standard Curve. Crystalline lead methyl mercaptide was used as the standard for methyl mercaptan. A sample was accurately weighed and appropriate dilutions made so that the final solution contained 150 γ of lead methyl mercaptide [Pb(SCH₃)₂] per ml. Various quantities of this solution (0.2 to 2.8 ml.) were added to the Folin-Wu trapping tubes and 5% mercuric acetate added to a total volume of 6 ml. The color was developed in the usual manner and the absorbance read against distilled water in a photoelectric spectrophotometer at a wave length of 500 mμ. Appropriate reagent blank corrections were made and the corrected absorbance values were plotted against actual methyl mercaptan concentration on rectilinear paper. The absorbance of the red complex follows Beer's law. Concentrations of methyl mercaptan between 5 and 110 γ may be determined by this procedure which corresponds to absorbance values between 0.05 and 0.80 with a 21-mm. light path.

Preparation of Crystalline Lead Methyl Mercaptide. The need for relatively stable complexes of methyl mercaptan became apparent during the development of a standard for colorimetric methods. Mercury, cadmium, copper, nickel, and zinc complexes of methyl mercaptan were prepared, but the complexes were found to be either relatively unstable, too insoluble for the acid conditions of the above mentioned colorimetric method, or they could not

be obtained in a purified form with constant composition.

Preliminary work on the development of a standard for methyl mercaptan indicated that the lead salt could be obtained in a relatively stable crystalline form. The literature reports on the lead salt of methyl mercaptan are very scanty. Wertheim (*8*) reported that the lead salt of methyl mercaptan precipitated as a gummy mass and was unsuitable for identification purposes.

In the authors' laboratories lead methyl mercaptide was prepared, crystallized, and its composition verified by an elemental analysis. The procedure used was as follows:

Bubble commercial purified gaseous methyl mercaptan through 250 ml. of a 10% lead acetate solution for 30 minutes. Collect the precipitate by suction filtration, air dry it, and then dry it in a vacuum desiccator. Add 5 grams of this dried material to 600 ml. of 5*M* sodium hydroxide and stir for 30 minutes. Filter the solution through asbestos to remove the brownish black turbidity.

Table I. Elemental Analysis of Lead Methyl Mercaptide

	Pb, %	S, %	C, %	H, %
Calculated	68.75	21.27	7.97	2.01
Actual[a]	69.0	20.9	8.00	2.06

[a] Lead and sulfur analysis made by Phoenix Chemical Laboratory Inc., Chicago, Ill. Carbon and hydrogen analysis made by Micro-Tech Laboratories, Skokie, Ill.

Collect the clear filtrate and add to it concentrated hydrochloric acid, slowly, with stirring (a Teflon-coated bar magnetic stirrer was used). Cool the container at intervals by immersing in cold water. Adjust the final pH of the solution to pH 7 and collect the bright yellow crystals by suction filtration. Recrystallize by the same procedure and dry the crystals overnight in a vacuum oven at a temperature of 45° C.

The crystals are fine rectangles which melt with decomposition at 165–6° C. Elemental analysis verified the compound to be essentially pure lead methyl mercaptide (Table I).

As a standard for methyl mercaptan the compound is relatively stable, and the color development is quantitatively the same over a period of several months if the crystals are kept well stoppered in a brown bottle.

Results with Irradiated Meat. Preliminary experiments indicated that 20 grams of fresh-ground irradiated meat was adequate for the assay of methyl mercaptan (CH₃SH). The results of the assay are expressed as micrograms of methyl mercaptan per gram of meat. The time required to ebulliate the meat samples with nitrogen was checked along with the temperature of the meat slurry during ebullition. A number of irradiated meat samples were ebulliated for 2, 4, and 6 hours at temperatures of 37°, 45°, 50°, 58°, and 65° C. From the experimental data obtained (Table II), a 4-hour ebullition period at 58° C. was chosen for the routine analysis of irradi-

---

Table II. Effect of Time and Temperature on Amount of Methyl Mercaptan from Gamma-Irradiated Ground Raw Beef

(Irradiated at 8 × 10⁶ rep)

Sample No.	Temp. of Meat during Ebullition, ° C.	Time, Hr.			
		0	2	4	6
			γ/G. Meat		
1	37	0.05	0.80	1.28	1.90
2	45	0.05	1.20	1.85	2.10
3	50	0.10	1.60	2.00	2.30
4	58	0.07	2.15	2.60	2.58
5	65	0.12	2.45	2.55	2.67

Table III. Influence of Gamma-Irradiation Dosage on Amount of Methyl Mercaptan from Ground Raw Beef

Sample	Dosage × 10⁶ rep								
	0	0.5	1	1.5	2	2	6	8	10
					γ/G.				
1	0.10	0.40	0.75	1.05	1.48	...	...	...	...
2	0.00	...	...	...	0.80	1.95	2.70	3.45	4.75
3	0.00	...	0.60	...	1.50	2.20	3.50	5.00	6.10
4	0.05	...	...	...	1.18	1.40	2.75	3.63	4.05
5	0.20	0.65	...	...	1.20	2.10	...	3.18	...
6	0.00	0.20	...	...	0.75	1.83	...	2.70	...
7	0.05	...	...	...	2.03	2.85	4.05	5.75	6.80
8	0.00	0.35	...	...	0.70	1.15	...	2.05	...
9	0.05	...	...	...	0.95	2.25	3.50	4.13	5.15
10	0.10	...	...	...	1.00	2.15	...	3.30	...
11a[a]	0.20	...	...	...	1.50	2.43	...	4.05	...
11b[a]	0.15	...	...	...	1.60	2.46	...	4.13	...

[a] Individual determinations. All other values are averages of duplicate determinations.

ated meat samples. At lower temperatures, the time required to ebulliate all the mercaptan was too long for convenient routine assay. At higher temperatures, the possibility of inaccurate high values as the result of protein degradation appeared likely.

A series of experiments was run to determine the effect of short storage periods on the volatile methyl mercaptan content of irradiated beef. No significant difference in the amount of methyl mercaptan was noticed within 36 hours after irradiation, if the irradiated samples were stored in the frozen state at −18° C. Thus, as long as the irradiated samples were kept frozen, methyl mercaptan could be quantitatively determined within 36 hours after irradiation.

On the basis of methyl mercaptan determinations on a large number of irradiated-meat samples, the methyl mer-

captan content of beef increases directly with gamma-irradiation dosage (Table III). In addition to this dosage effect, there is a difference in the amount of methyl mercaptan formed in different samples of beef during irradiation.

Literature Cited

(1) Almy, L. H., *J. Am. Chem. Soc.* **47**, 1381–90 (1925).
(2) Batzer, O. F., Doty, D. M., *J. Agr. Food Chem.* **3**, 64–7 (1955).
(3) Fischer, E., *Ber.* **16**, 2234–6 (1883).
(4) Herk, L. P., Jr., Levy, E. J., O'Brien, D. C., Stahl, W. H., "Identification of Certain Constituents of Odor of Irradiated Meat," Division of Agricultural and Food Chemistry, 130th Meeting, ACS, Atlantic City, N. J., September 1956.
(5) Marbach, E. P., Doty, D. M., *J. Agr. Food Chem.* **4**, 881–4 (1956).
(6) Sands, A. E., Grafius, M. A., Wainwright, H. W., Wilson, M. W., U. S. Dept. Interior, Bur. Mines, Rept. Invest. 4547 (1949).
(7) Snell, F. D., Snell, C. T.. "Colorimetric Methods of Analysis," Vol. **I**, Van Nostrand, New York, 1936.
(8) Wertheim, E., *J. Am. Chem. Soc.* **51**, 3661–4 (1929).

FOOD TANNINS MEASUREMENT

Determination of Food Tannins by Ultraviolet Spectrophotometry

JOSEPH L. OWADES, GEORGE RUBIN, and M. W. BRENNER

Schwarz Laboratories, Inc., Mount Vernon, N. Y.

The need for simpler and more accurate methods for the quantitative determination of tannins prompted the development of a direct procedure based on the ultraviolet absorption of these compounds. The tannins are extracted with ethyl acetate, and, after evaporation of solvent, are taken up in acidified methanol. The absorbance of the methanol solution is measured at 270 mμ. The method was developed for tea and beer, but may be applicable to other materials as well.

AVAILABLE METHODS for the determination of the heterogeneous group of compounds best described as tannins have been reviewed (*10*), and two of the more popular ones critically compared (*15*). The latter are the permanganate titration procedure of Loewenthal [the official method for tannins in tea, coffee, and spices (*7*)], and the colorimetric Folin-Denis-Pro procedure [official for distilled liquors (*2*)]. The limitations of these methods are well known.

The tannins have a common distinguishing feature—namely, the presence of two (generally) oxygenated aromatic rings, joined by a three-carbon chain of varying oxygen substitution (*5*). The method for determining tannins proposed here, which takes advantage of this

common structure, is not subject to interference by aliphatic reducing substances, and is unique among the many methods for estimating tannins.

This procedure involves extracting the tannins from an acidified sample by ethyl acetate, removing the solvent, dissolving the residue in methanol, acidifying the solution, and determining the absorbance at the wave length of maximum absorption (270 mμ for beer, 275 mμ for tea). A prior extraction of the sample with a nonpolar solvent (such as iso-octane) may be necessary with some materials. The tannin content of the sample is calculated from the extinction coefficient of the tannins present. If this value is not known, the foodstuff or other material to be analyzed is extracted as for the determination—

but on a scale that allows isolation of the tannins and determination of the extinction coefficient.

Procedure

Reagents and Apparatus. Ethyl acetate, reagent grade, redistilled.
Hydrochloric acid, 1 N.
Iso-octane.
Sodium sulfate, anhydrous, reagent grade.
Beckman spectrophotometer, Model DU.

Isolation of Tannins from Hops. Twenty grams of dried hops was homogenized with benzene in a Waring Blendor (*16*), and extracted by decantation through a filter paper with about 3.5 liters of benzene—or to the disappearance of color. The hops were then extracted in a similar manner with 2.5