to a pyridoxal standard appears to be equal to or slightly less than that for *Saccharomyces carlsbergensis*. What activity for humans the unknown form of vitamin B_6 may possess is not known. That it has considerable is suggested by the fact that evaporated milk has been used in infant formulas for many years without the appearance of deficiency symptoms and thus appears to possess adequate vitamin B_6 activity to meet the requirement of the human infant.

Summary

Modifications of the *Neurospora sitophila* 299 and *Saccharomyces carlsbergensis* methods of assay for vitamin B_6 give similar but not identical results when applied to fresh or pasteurized milk or to nonfat dry milk solids, but different results when applied to evaporated milk, particularly after storage. The *Neurospora sitophila* method gives the higher results and indicates a vitamin B_6 content similar to that of fresh milk. The *Saccharomyces carlsbergensis* method usually gives lower results, which suggests loss or change of the vitamin B_6 content.

In the sterilization of evaporated milk, pyridoxal is partially converted to pyridoxamine. This conversion probably continues during storage.

During the storage of evaporated milk a part of its vitamin B_6 activity appears to be changed to an unknown form.

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FOOD IRRADIATION

Sulfides Released from Gamma-Irradiated Meat as Estimated by Condensation with *N,N*-Dimethyl-*p*-phenylenediamine

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The reaction of hydrogen sulfide with *N,N*-dimethyl-*p*-phenylenediamine to form methylene blue has been adapted to determine small quantities of hydrogen sulfide from gamma irradiated meat. The odorous vapors from irradiated meat are carried with a stream of nitrogen into a trapping tube containing cadmium hydroxide and sodium hydroxide. The color is then developed by adding a mixture of the acid amine solution and Reissner's solution to a cup in the trapping tube, closing, and shaking. The intensity of the methylene blue color developed is then measured in a photoelectric colorimeter at 665 mμ. The method can be used for the quantitative estimation of 2 to 16 γ of hydrogen sulfide. Experiments show that free hydrogen sulfide probably is not present in gamma irradiated meat but that cadmium sulfide is formed in the trapping solution from some volatile sulfur-containing complex.

BEFORE APPLYING IONIZING RADIATIONS for the cold sterilization of certain meats, a method to prevent the development of undesirable odor and flavor must be found. Batzer and Doty (*2*) reported that hydrogen sulfide was probably one of the components of the undesirable odor developed in meat during irradiation. To study the effect of various conditions and methods of treatment on the development of hydrogen sulfide during gamma irradiation of meat, a selective, sensitive method for the estimation of small quantities of hydrogen sulfide was devised.

The classical reaction between hydrogen sulfide and *N,N*-dimethyl-*p*-phenylenediamine to form methylene blue, first proposed by Emil Fischer (*6*) in 1883, was the basis for the improved quantitative method. Since then several

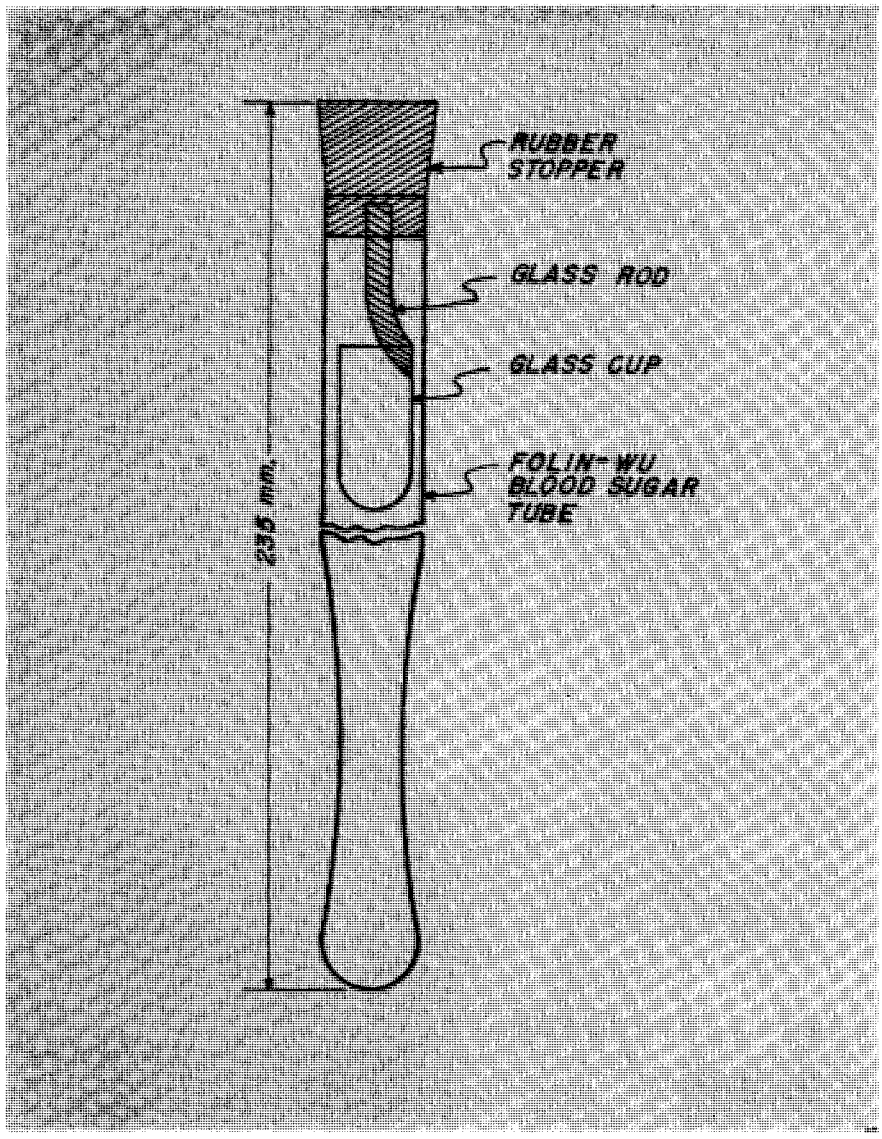

Figure 1. Folin-Wu blood sugar tube for trapping effluent vapors with glass cup for mixing color reagents

investigators have adapted the reaction and studied the variables for the determination of hydrogen sulfide in various substances (*7*, *8*). Almy (*1*) and Sheppard and Hudson (*10*) applied the test to hydrogen sulfide evolved from biological samples. The former author used 0.6% zinc acetate, while the latter used alkaline zinc acetate to absorb the hydrogen sulfide. Diemair, Strohecker, and Keller (*5*), used the reaction to determine the hydrogen sulfide evolved from biological materials. However, they did not use a trapping solution but absorbed the hydrogen sulfide directly in the color-forming reagents.

Burton (*4*) and Sands and coworkers (*9*) applied the methylene blue reaction to the determination of hydrogen sulfide in gases. Burton used alkaline zinc acetate as a trap for the hydrogen sulfide while Sands and coworkers used 2% zinc acetate. Budd and Bewick (*3*) used the methylene blue reaction to determine the sulfides and reducible sulfur in alkalies. They used 2% zinc acetate as the trapping solution.

Table I. Apparent Amount of Hydrogen Sulfide Recovered from Sodium Sulfide in Closed and Open Trapping Tubes

Procedure	H_2S, γ			Recovery, %
	I	II	Av.	
Closed tube as described	5.22	5.20	5.21	100
Open tube. Acid amine solution added followed by Reissner's solution	5.10	5.05	5.08	97.5
Open tube. Reissner's solution added followed by acid amine solution	4.82	5.17	5.00	96.0
Open tube. Acid amine and Reissner's solution mixed and added	4.95	4.90	4.93	94.6

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 0.03 or less at 500 mμ with a 16-mm. path length. When protected from light, the solution is stable indefinitely.

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 indefinitely.

Cadmium Hydroxide Suspension. Add sodium hydroxide solution (ca. 4*N*) in excess of a cadmium acetate solution and centrifuge down the white precipitate of cadmium hydroxide. Decant the supernatant, suspend the precipitate in distilled water, and again centrifuge down the cadmium hydroxide. Repeat the washing procedure until the pH of the cadmium suspension drops to 9.6. Suspend the washed, wet precipitate in sufficient distilled water to make approximately 0.1*N* cadmium hydroxide suspension (determined by acid titration).

Procedure

The procedure for determining the hydrogen sulfide from the volatile sulfur components from meat is as follows: Spray some antifoam A into a 30 × 200 mm. heavy-walled test tube. Place in the tube 5 grams of finely ground meat, 50 ml. of distilled water, and a glass rod about 8 mm. in diameter and 30 mm. long. Disperse the meat thoroughly by shaking the test tube. Fit the tube with a two-hole rubber stopper; in one hole insert a 6-mm. (outside diameter) glass tube so that its end is within 5 mm. of the bottom of the tube; in the other hole insert a short piece of glass tubing for a Tygon tubing connection to the trapping tube.

Place the tube in a water bath at 65° C. and connect immediately to the nitrogen supply and the trapping tube. The trapping tube is a Folin-Wu sugar tube graduated at 25 ml. and contains 5 ml. of 0.1*N* cadmium hydroxide suspension and 1 ml. of 0.1*N* sodium hydroxide. 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 2 to 3 mm. (outside diameter). Immerse the trapping tube in ice water to keep the trapping solution at 0° to 5° C.

Allow commercial high purity, water-pumped nitrogen to bubble through the meat slurry at a rate of 0.5 cubic foot per hour. After 2 hours of ebullition, disconnect the trapping tube, break off the capillary tip, and add it to the trapping tube (to retain the cadmium sulfide precipitate on the tip). Add 1.5 ml. of the amine solution and 0.5 ml. of Reissner solution by means of a special cup (Figure 1). Stopper, invert, and shake the tube for 10 minutes to mix the reagents. Rinse the special cup two or three times, each time allowing the rinse water to flow into the tube. Dilute to 25 ml. with distilled water, shake, and allow to stand 30 minutes at room temperature. Transfer a portion of the solution to a spectrophotometer tube and read against a distilled water blank at 665 mμ. Deduct from the reading the absorbance of a reagent solution containing no sulfide. Read the amount of hydrogen sulfide present from a standard curve prepared from data obtained by using solutions containing known amounts of sulfide (see below).

Standard Curve. Wash a large crystal (about 1 gram) of sodium sulfide nonahydrate with distilled water until just the core (about 0.1 gram) remains. Dissolve in distilled water and dilute to 1 liter. Standardize this solution by the usual iodine-thiosulfate method using 100-ml. aliquots. Add aliquots of this standardized solution (0.1 to 1.0 ml.) to the alkaline cadmium hydroxide suspension in a series of Folin-Wu tubes, develop the color as described above, and read the absorbance values in a suitable spectrophotometer at 665 mμ. After subtracting the reagent blank value, plot the readings against sulfide concentration on rectilinear paper.

Although a straight-line relationship is obtained under the conditions of color development described here, dilution of the colored solution with properly diluted reagents does not result in an equivalent reduction in absorbance. The diluted solutions are more intensely colored than expected (about 10% greater absorbance than expected at 50% dilution).

Under the conditions described here the range for the quantitative estimation of hydrogen sulfide is 2.0 to 16.0 γ, which corresponds to absorbance values between 0.1 and 0.8 with a 16-mm. light path.

Results

Use of a Sealed Trapping Tube. Although other investigators have developed the methylene blue color from hydrogen sulfide in an open tube, preliminary experiments indicated that there was some loss of hydrogen sulfide when the acid amine solution was added under these conditions. To prevent this a special trapping tube was designed. It consisted of a Folin-Wu sugar tube that contained the alkaline cadmium hydroxide, a sulfur-free stopper, and a glass cup attached to the stopper by a glass rod (Figure 1). When the color was developed in this closed tube assembly with the special cup for adding the reagents, greater color development was obtained than with an open tube (Table I).

Trapping Agents. Since some components in meat juice were shown to prevent the condensation of the amine with hydrogen sulfide to form methylene blue, the volatile components must be removed from the meat and used in the condensation reaction. The color-developing reagents could not be used as a trapping solution for the volatile

Table II. Loss of Hydrogen Sulfide When Sodium Sulfide Is Added Directly to Trapping Solutions at 10° C.

(Approximately 3.5 γ H_2S present)

Trapping Solution	pH	Time, Hr.	Loss, % No nitrogen	Loss, % In nitrogen stream
0.1*N* NaOH	13	0	0	
		24	24	11
0.025*M* sodium Veronal		0	0	
	9.8	96	40	
1% cadmium chloride	5.5	0	0	
		24		56
5% cadmium acetate in 25% acetic acid		0	0	
	3.8	24		39
		48		56
0.5% lead acetate	6.5	0	33	
5% zinc acetate in 1% sodium acetate solution		0	0	
	6.5	24	2	50
2% cadmium acetate		0	0	
	6.5	24	9	13
2% cadmium acetate in 1% sodium carbonate		0	0	
	9.8	24		30
0.5% uranium acetate		0	0	
	6.5	24		80
Saturated cadmium hydroxide		0	0	
	9.6	24	0	2
		48	0	
5 ml. 0.1*N* cadmium hydroxide		0	0	
1 ml. 0.1*N* sodium hydroxide	13	24	0	0

Table III. Hydrogen Sulfide Released from 5.0 Grams of Ground Beef with and without the Addition of Sodium Sulfide before or after Irradiation in Co^{60} Source

Sample	Radiation Dosage, 10^6 Rep		H_2S Added (as Na_2S), γ Before irradiation	H_2S Added (as Na_2S), γ After irradiation	H_2S Released, γ
A	0		0	...	1.8
		I	0	0	16.4
A	4.3	II	0	0	19.7
		Av.			18.0
		I	12.7	0	19.7
A	4.3	II	12.7	0	19.7
		III	12.7	0	17.6
		Av.			19.0
B	0		0		0.4
B	0		6.4		0.8
		I	0	0	10.3
B	4.3	II	0	0	9.8
		Av.			10.1
		I	0	6.4	12.1
B	4.3	II	0	6.4	13.5
		Av.			12.8

Table IV. Hydrogen Sulfide Released from Sodium Sulfide Solutions

1 Ml. Freshly Prepared Na_2S Solution	H_2S Recovered, γ	Recovery, %
Added directly to trapping solution	7.1	100
	7.1	100
Held for 2 hours at room temperature	6.3	89
	6.3	89
Added to 50 ml. distilled water	2.4	34
	3.1	43
	2.8	39
Added to 50 ml. boiled distilled water	3.1	43
	2.0	28
	3.3	46

sulfur components because they were sensitive to hydrogen sulfide for only 5 to 10 minutes, while 2 to 24 hours (depending upon the temperature of ebullition) was required to remove the odor components completely from irradiated meat. Thus a special trapping solution was used to collect the volatile sulfur components.

When the type of trapping solution to be used was considered it was recognized that a solution of pH 6.5 or lower would probably allow considerable loss of sulfide, since hydrogen sulfide would exist predominantly in the undissociated form at low pH levels. On the other hand, at pH 11 or higher, hydrogen sulfide would be present predominantly in the dissociated form; in dilute concentrations the sulfide ion is readily air oxidized to disulfides that will not undergo the methylene blue conversion. It was thought, therefore, that precipitation of the sulfide ion with a heavy metal salt at high pH should prevent sulfide loss and still allow the methylene blue conversion.

To check these ideas several trapping solutions were tested and the loss of reactive hydrogen sulfide after various time intervals was determined. The data (Table II), in general, confirm the ideas presented above. At pH 6.5 or below sulfide was lost during the nitrogen ebullition; for the lead acetate trap, the lead sulfide was so insoluble that only two thirds of the sample would dissolve in the acid conditions for the methylene blue conversion. Saturated cadmium hydroxide gave the best results. The addition of sodium hydroxide was necessary also for irradiated meat samples to prevent a drop in pH of the trapping solution from the carbon dioxide carried over. One milliliter of 0.1*N* sodium hydroxide adequately held the pH near 13, since cadmium sulfide, cadmium hydroxide, and cadmium carbonate are sparsely soluble at this pH.

Interfering Substances. High concentrations of electrolytes do not interfere with the reaction; however, all oxidizing agents tested and some reducing compounds, such as hydrogen iodide, do interfere. Mercaptans do not interfere. In fact, the same method can be utilized for quantitative determinations of mercaptans; in this case a red solution is formed. The lower sensitivity limit, however, is 50 γ of methyl mercaptan, and hydrogen sulfide interferes in so far as the methylene blue formed partially absorbs at the maximum absorption band (490 mμ) of the red complex.

Recovery Experiments. Known amounts of sulfide as sodium sulfide were added to both irradiated and nonirradiated meat and the sulfide in the samples was determined. The results (Table III) showed that only a fraction (6 to 42%) of the sulfide added to ground meat could be recovered as hydrogen sulfide by the procedure described here. This was true for both irradiated and nonirradiated meat; greater recovery of added sulfide was observed when the sodium sulfide was added after irradiation.

To shed some light on the reason for the poor recovery of sulfide from ground meat, recovery of sulfide from distilled water by nitrogen ebullition was investigated. One milliliter of a sodium sulfide solution containing the equivalent of 7.1 γ of hydrogen sulfide was added to tubes containing 50 ml. of distilled water or boiled distilled water which had been connected to the ebullition system and purged with nitrogen for 1 hour to remove all air from the tubes and apparatus. The ebullition and hydrogen sulfide determinations were then performed as previously described. The results (Table IV) show that only about 30 to 40% of the sulfide was recovered from either distilled or boiled distilled water. Furthermore, the hydrogen sulfide equivalent of the original sodium sulfide solution dropped approximately 11% on standing 2 hours at room temperature. Since recovery of sulfide from solution or meat is neither complete nor reproducible, the ebullition procedure cannot be used to recover sulfide quantitatively from solution.

These results suggest that hydrogen sulfide is lost from solution or meat slurries by oxidation, as experiments have repeatedly shown that no additional hydrogen sulfide is released by ebullition beyond 2 hours at 65° C. or upon the addition of sulfuric acid to the solution or meat slurry. Thus it appears unlikely that the hydrogen sulfide obtained from irradiated meat actually is present as free hydrogen sulfide in the meat; however, a complex of hydrogen sulfide and an unknown substance, which is volatile and not readily oxidizable, may be present. This volatile sulfide complex, which might be one of the off-odor constituents of irradiated meat, could react in the cadmium hydroxide trap to form cadmium sulfide which then formed the methylene blue color. The procedure described here, then, is not quantitative for hydrogen sulfide present in irradiated meat but is rather for sulfides formed from the volatile constituents, or volatile sulfides in non-oxidized form, present in the meat.

The method, then, offers one of the few objective means for determining the extent of undesirable changes that occur in meat subjected to ionizing radiations for sterilization.

Preliminary Results with Irradiated Meat. On the basis of preliminary data, some of which were obtained during the development of the method, it appears that 1 to 4 γ of hydrogen sulfide per gram of meat is formed from the volatile components of ground beef that has been irradiated at a dosage level of 2,000,000 to 4,000,000 rep. After irradiation at 10,000,000 rep, only about half this amount of hydrogen sulfide is released. If ground beef is frozen and stored at 0° F. before irradiation, the amount of hydrogen sulfide decreases with storage time for at least 2 weeks. Less hydrogen sulfide is released from ground beef of high fat content (20%) than from beef containing less fat (<10%) at the same irradiation dosage level.

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