

Determination of Free and Combined Formaldehyde Using Modified Chromotropic Acid Procedure Application to Determination of Piperine Content of Pepper

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A modified colorimetric chromotropic acid procedure has been developed for the quantitative determination of formaldehyde liberated from the methylenedioxy group in piperine, the principal substance associated with the sharp taste of pepper. The method thus provides a simple, rapid procedure for the analysis of the piperine content of pepper. The conditions selected are also advantageous for the determination of free or combined formaldehyde in other compounds.

VERY little work has been published on the specific determination of piperine, which probably is the major pungent component of pepper (*Piper nigrum*). Use has been made of such general methods as Kjeldahl nitrogen estimation (15) or the weighing of a partially purified residue following extraction of the ground spice (8). Fagen, Kolen, and Hussong (8) have recently reported an ultraviolet spectrophotometric method for

the determination of piperine in oleoresin of pepper. Their method is both specific and sensitive, but cannot be used by laboratories that have only visual range photometers.

The desire by the spice trade and other segments of the food industry for a simple, rapid method for the determination of piperine led to an investigation of the possibility of developing such a method.

Piperine forms colored complexes with a number of alkaloidal reagents (2) and colored salts with some concentrated acids (2, 13). None of these, however, was found useful for a quantitative colorimetric procedure; reactions were qualitative, or colors were unstable, or absorption maxima were located in the ultraviolet region, just beyond the range of several commonly used photoelectric colorimeters. Attempts to take advantage of the weakly basic properties of piperine (which is the piperidide of the unsaturated piperic acid) were not successful. The amido linkage could not be titrated, even in nonaqueous systems.

However, piperic acid contains a methylenedioxy group, which should yield formaldehyde on acid hydrolysis. Bricker and Vail

(5) found that the purple pigment derived from reaction of chromotropic acid with formaldehyde in strong acid (7) was obtained with piperine, among several other compounds containing combined formaldehyde groups. According to Beroza (3), whose work was published while the present work was in progress, the yield of formaldehyde from piperine is not quantitative, either by the original Bricker and Johnson procedure (4) or by Beroza's modified method.

The chromotropic acid method described below gives the theoretical yield of formaldehyde when applied to piperine. In addition, the sensitivity of the reaction has been increased and the mechanics of the procedure have been simplified.

REAGENTS AND APPARATUS

Chromotropic acid, sodium salt (4,5-dihydroxy-2,7-naphthalenedisulfonic acid, disodium salt). A 25% aqueous solution is prepared fresh daily by dissolving the salt in warm distilled water. Sometimes filtration through a sintered disk or centrifugation is necessary to remove a black sediment from the solution.

Piperine. A pure sample of piperine was prepared from ground black pepper (11) and recrystallized several times from ethyl alcohol to a constant melting point (129–129.5° C.). A commercial sample of piperine, obtained from the Amend Drug Co., New York, was also used after two recrystallizations from ethyl alcohol. The purity of these materials was confirmed by the ultraviolet spectrophotometric method (8), using a Cary recording spectrophotometer.

Sulfuric acid, reagent grade, concentrated (96%).

Ethyl alcohol, 95%. Denatured alcohol S.D. 30 may be substituted.

Glass-stoppered 25-ml. volumetric flasks, borosilicate glass.

Beckman Model DU spectrophotometer with 1-cm. cells. A slit width of 0.03 mm. was used throughout.

PROCEDURE

Sample Preparation. Weigh accurately about 0.5 gram of ground pepper and transfer to a 250-ml. volumetric flask, using 95% ethyl alcohol as a wash liquid. Add about 100 ml. of ethyl alcohol to the flask and boil 3 hours on the steam bath with occasional swirling. Cool and dilute to the mark with 95% ethyl alcohol. Filter or centrifuge a few milliliters of the extract. (The small volume occupied by the alcohol-insoluble material does not cause a significant error. In fact, samples as large as 3 grams can be handled without loss in accuracy.)

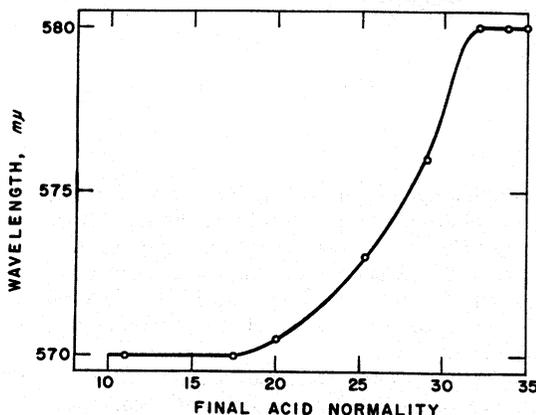


Figure 1. Change in wave length of maximum absorbance with increasing sulfuric acid concentration

Determination. Pipet 1-ml. aliquots of the filtrate into each of two 25-ml. volumetric flasks, A and B. Add 1 ml. of ethyl alcohol to a third flask, C. Add 0.5 ml. of chromotropic acid solution to flasks A and C. Pour 10 ml. of concentrated sulfuric acid from a graduate into each of the three flasks, swirling to mix the contents. Some heating takes place, but the alcohol barely reaches the boiling point. Splattering does not occur because of

the shape of the flask, especially if it is tilted during the sulfuric acid addition. Heat the flasks in a boiling water bath for 30 minutes, stoppering loosely after 1 or 2 minutes.

Cool to room temperature and dilute to the mark with concentrated sulfuric acid (no heating occurs). Mix thoroughly and determine the absorbance of the solutions in flasks A and B at 580 mμ. Use flask C as the blank for A and concentrated sulfuric acid as the blank for B.

Table I. Effect of Sulfuric Acid Concentration Used for Dilution on Results of Piperine Determination

Piperine, μ Mole	Diluent	Final Volume, Ml.	Absorbance	Relative Absorbance, % ^a
0.5	Water	25	0.254	77 ^b
0.5	18N H ₂ SO ₄	25	0.264	80 ^b
0.5	24N H ₂ SO ₄	25	0.274	83
0.5	30N H ₂ SO ₄	25	0.294	89
0.5	34N H ₂ SO ₄	25	0.330	100
0.5	36N H ₂ SO ₄	25	0.331	100
0.5	36N H ₂ SO ₄	50	0.164	100
1.0	36N H ₂ SO ₄	50	0.330	100

^a Expressed as per cent of absorbance found for equivalent amounts of formaldehyde treated the same way. Absorbance measured at λ_{max} of each final acid concentration.

^b Erratic unreproducible results.

Calculation. The absorbance of 1 μ mole of piperine (or formaldehyde) in 25 ml. is 0.660. The per cent of piperine in the original sample is:

$$\begin{aligned} \% \text{ piperine} &= \left(\frac{A - B}{0.660} \right) \times \frac{\text{mol. wt.} \times 10^{-4} \times \text{diln. factor}}{\text{sample wt., grams}} \times 100 \\ &= 10.81 \left(\frac{A - B}{\text{sample wt., grams}} \right) \end{aligned}$$

where A = absorbance, flask A

B = absorbance, flask B

The absorbance of the solution without chromotropic acid (flask B) is due mainly to formation of a colored salt of the hydrolytic products of piperine with sulfuric acid. Although it varies slightly from day to day, the absorbance has an average value of 10% of the other solution (A). Therefore, for more rapid work with a slight loss in accuracy, flask B can be omitted. The calculation then is:

$$\% \text{ piperine} = 10.81 \left(\frac{0.9 A}{\text{sample wt., grams}} \right)$$

RESULTS AND DISCUSSION

The procedure above differs essentially from the original work of Bricker and Johnson (4) in only one detail: the final concentration of sulfuric acid.

Table I shows the effect of diluting reaction mixtures to volume with varying concentrations of sulfuric acid. Bricker and Johnson (4) suggested the use of 18N sulfuric acid for dilution of samples when acetone was present. In the case of piperine, however, no increase in absorbance occurs with 18N acid, but as the acid concentration is increased above 24N the absorbance increases to that obtained for formaldehyde solutions treated in a similar manner (Table I).

As the acid concentration is increased, the wave length of maximum absorbance shifts (Figure 1) and there is an increase in absorptivity. Measured at 580 mμ, the absorbance of the reaction mixture diluted with concentrated sulfuric acid is about 10% higher than that obtained after dilution with water. A recent note by Feigl and Hainberger (9) states that the sensitivity of Eegriwe's qualitative test for formaldehyde (7) can be greatly increased by reaction under anhydrous conditions. With the experimental procedure above, a plateau of color intensity is apparently reached, beyond which a substantial increase

in the amount of sulfuric acid does not affect absorbance (Table I). The use of more concentrated acid than prescribed by Bricker and Johnson (4) has no effect on the close adherence of the colored complex to Beer's law.

Dilution with sulfuric acid instead of water makes the analytical procedure simpler and faster. Exact dilution to the mark is a one-step process, because no heat is generated and no cooling or readjustment of volume is required. The color of the solutions diluted with concentrated acid is as stable as those diluted with water (4). The following data were obtained when 0.5 μ mole of piperine was diluted with 34*N* sulfuric acid:

Time after Dilution, Hours	Absorbance
0	0.330
0.5	0.330
3	0.329
24	0.329
48	0.331

The rate of absorption of water from the atmosphere is negligible, although it is recommended that prolonged exposure to the air be avoided, especially on humid days.

The data in Table II indicate that the standard deviation of the determination is 0.5%.

Extraction of Pepper. The efficiency of the alcohol extraction was determined by comparison with analyses of "nonvolatile ether extracts" obtained from continuous ether extraction of pepper samples, according to the AOAC procedure (1). Following extraction of pepper samples with ether, the solvent was evaporated and the residues were carefully dried to constant weight at 110° C. These residues, which are about 80% piperine (15), were dissolved in ethyl alcohol, made up to a definite volume, and analyzed by the above chromotropic acid procedure. The results are shown in Table III along with those obtained by the direct alcohol extraction method on portions of the same pepper samples.

Table II. Precision of Method

Sample	Piperine Found, %	Mean	Std. Dev., %
Piperine, 143 γ	99.7	99.85	0.52
	99.4		
	99.7		
	100.6		
Black pepper	6.94	6.98	0.43
	6.99		
	6.99		
	7.01		

As a further check on completeness of extraction, the residue from a 3-hour ethyl alcohol extraction was filtered off, washed with cold alcohol, and re-extracted for several hours with fresh alcohol on the steam bath. A qualitative test with chromotropic acid was negative, even after fivefold concentration of the filtrate.

Other Applications. A sample of isosafrole (practical grade, purity unknown) analyzed 92 to 95% with the present procedure. An increase in the amount of chromotropic acid per sample of isosafrole, as suggested by Beroza (3) for certain compounds, was not tried.

Alcoholic piperonal solutions, which give theoretical amounts of formaldehyde by both the original Bricker and Johnson procedure and by Beroza's modification (3) gave erratic, less than quantitative recoveries by the present procedure, until it was

noticed that a precipitate formed during the reaction period. Apparently, piperonal is insoluble in concentrated sulfuric acid. Use of less sulfuric acid—i.e., increasing the ratio of solvent to sulfuric acid—during the heating period permitted maximum pigment formation.

Formaldehyde solutions, resublimed trioxymethylene, and diethoxymethane (formaldehyde diethylacetal) all gave theoretical color yields, with the advantages of higher absorptivity at 580 $m\mu$ and greater ease of manipulation, as described above. Diethoxymethane is recommended as a standard for the method. It is easily obtainable pure and is water-soluble, giving stable solutions.

Table III. Comparison of Chromotropic Acid Determination of Piperine after Continuous 20-Hour Ether Extraction or Rapid Ethyl Alcohol Extraction

Sample	Time of Alcohol Extraction, Hours	Piperine Found, %	
		Ether	Ethyl alcohol
Lampong pepper	3	7.05	7.02
	3	7.04	7.03
Sarawak pepper-1	3	6.37	6.37
Sarawak pepper-2	2	6.59	6.30
	3		6.61
	18 ^a		6.60

^a Continuous extraction in Soxhlet apparatus.

General Comments. The method suffers from the fact that the equivalent weight of formaldehyde is small compared to that of piperine; this disadvantage is offset, however, by the high sensitivity of the reaction.

When the method is used on pepper extracts, it is not entirely specific; chavicine and other isomers of piperine which are present in pepper are included in the results as piperine. These compounds are present only in small amounts in pepper (10, 12, 14), so their inclusion in the piperine determination as a measure of pungency leads to only a minor inaccuracy, because they also contribute (11) to the pungency of the spice.

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