

A Critical Study of Two Procedures for the Determination of Piperine in Black and White Pepper

CHRISTIANE GENEST,
D. MORISON SMITH, and
D. G. CHAPMAN

Food and Drug Directorate, Department of National Health and Welfare, Ottawa, Canada

The apparent piperine contents of black and white peppers were determined by both a spectrophotometric method and a colorimetric procedure. The piperine contents by the latter procedure were consistently higher than that indicated by the ultraviolet measurements. The variables causing these discrepancies in results were investigated using column fractionation of pepper extracts as well as by paper and thin-layer chromatography. Analogs of piperine with less than two conjugated ethylenic linkages could not be found in pepper extracts. However, all black and white peppers contained the piperine analog with three conjugated ethylenic linkages, piperettine. Measurements of the ultraviolet absorbances at 343 $m\mu$ for piperine and at 364 $m\mu$ for piperettine and calculation of the ratio of these two biochemically related constituents gave results for true piperine and piperettine contents which served to some extent as an indication of the geographical origin of a pure pepper.

THE ACCURATE ANALYSIS of piperine, 1-piperoylpiperidine, in black and white pepper (*Piper nigrum*, L.) has been undertaken to provide additional information on the components of natural foods which are responsible for their commercial value. In addition to augmenting the determination of the non-specific criteria, such as the nonvolatile ether extract, pepper starch, crude fiber, total ash, and ash insoluble in hydrochloric acid, the analysis should enable one to determine the geographical origin of pure peppers of unknown provenance, the constitution of blends, and, most important, the recognition of the extent and nature of adulteration.

Piperine, the major constituent of the bite principle in black and white pepper can be determined by various chemical methods (1, 4, 9).

The first method to be used was the determination of total nitrogen in the nonvolatile ether extract of ground pepper and then use of calculations to obtain the piperine content (7). Some doubts have already been cast upon the accuracy of this method since it analyzes for any ether-soluble, nonvolatile, nitrogenous substance (4, 16), in addition to piperine.

The colorimetric method (9) adopted by the American Spice Trade Association (A.S.T.A.) (17) for laboratories equipped with colorimeters measures the intensity of a purple color which is formed by the quantitative reaction of formaldehyde with chromotropic acid in the presence of concentrated sulfuric acid. One equivalent of formaldehyde is liberated by the sulfuric acid hydrolysis of the methylenedioxy grouping in piperine.

The spectrophotometric method (4) is based on absorption in the ultraviolet

region at 345 $m\mu$ by the piperine in oleoresin of black and white pepper.

All three chemical methods use an organic solvent for the extraction of the alkaloid, the solvent being either evaporated off or used directly for the analysis. This pepper extract is used for the analysis without purification. Differences between results for piperine obtained by various methods might then be caused by a given method being sensitive to material which was not piperine. High results are suspect. Various techniques were used to investigate these interfering materials. They were fractionated by column chromatography, separated into individual spots by paper and thin-layer chromatography, and compared with synthetic analogs of piperine of lower molecular weight. The fractions collected from the column, the pure synthesized compounds, and the spots after elution were examined using infrared and ultraviolet spectrophotometry as well as the A.S.T.A. colorimetric procedure. A simple analytical method has been developed for the determination of both piperine and piperettine (14) which was found to be present in all black and white peppers.

The present paper discusses the shortcomings of the colorimetric A.S.T.A. procedure and assesses the comparative validity of the spectrophotometric ultraviolet method in the analysis of black and white peppers for piperine.

Experimental

Apparatus. Beckman Spectrophotometer Model DU with ultraviolet attachment and photomultiplier tubes.

Cary Recording Spectrophotometer, Model 14.

Perkin - Elmer Spectrophotometer Model 221.

Glass column apparatus with reservoir, o.d. 25 mm., length 320 mm. (12).

Thin-layer chromatograph (Research Specialties Co.).

Ultraviolet light source, 366 $m\mu$.

Chromatographic jars—cylindrical: i.d. 6 inches, height 12 inches; and rectangular: 9½ × 9 × 4½ inches.

Microliter pipets.

Glass plates, 8 × 8 inches.

Whatman 3MM paper, 11 × 11 inches.

Reagents. Pyridine, reagent grade (USP).

Florisil 60 to 100 mesh, activated for 2 hours at 300° C.

Activated Carbon Darco G-60.

Silica Gel G (Research Specialties Co.).

Piperine, alkaloid, USP.

Diethylamine.

Silicone 550 (fluid).

Diethoxymethane.

Chromotropic acid, sodium salt.

Procedures. PIPERINE STANDARD. Samples of piperine were treated with activated carbon and twice recrystallized from ethyl acetate or recrystallized from ethanol without adding charcoal. Light, yellow crystals were obtained in both cases, m.p. 132° C. The products were spectrophotometrically identical in the ultraviolet. Standard solutions were stored in brown flasks and proved to be stable for at least a month.

EXTRACTION. An 0.8-gram sample of pepper, ground to pass a U. S. standard 20-mesh sieve, was extracted in 100 ml. of 95% ethanol using 250-ml. volumetric flasks covered with black masking tape to protect the pepper solution from the influence of light (17). A funnel, put into the neck of the flask, prevented excess evaporation of solvent.

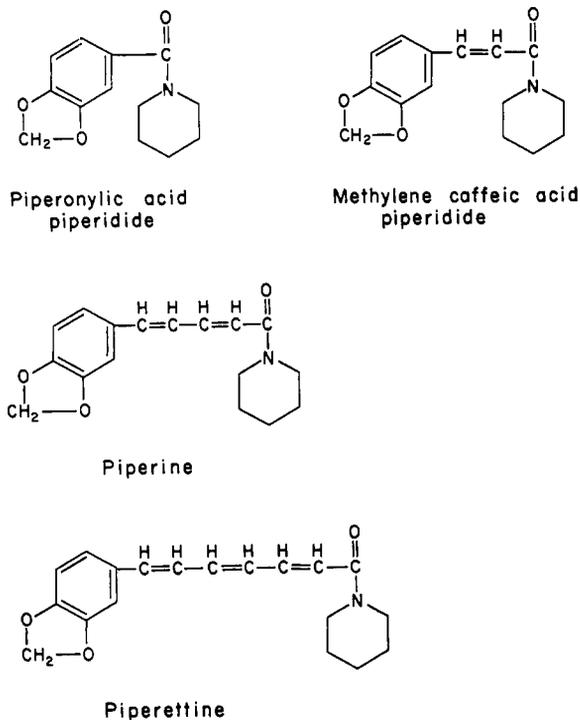


Figure 1. Analogs of piperine

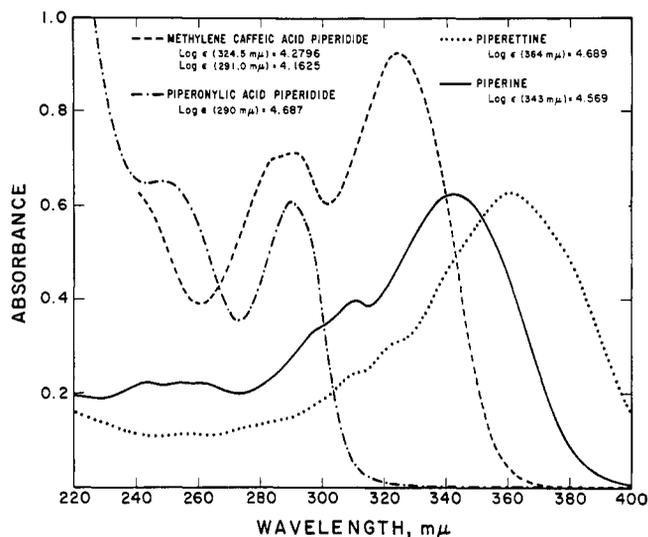


Figure 2. Ultraviolet spectra of piperine and its analogs at convenient concentrations

COLORIMETRIC METHOD. The A.S.T.A. method (9, 17) was applied to all samples under investigation with some minor changes. The chromotropic acid was recrystallized before use. For this purpose, 10 grams of the acid were dissolved in 100 ml. of distilled water, and about 10 grams of decolorizing charcoal were added. The material was then processed according to Bricker and Johnson (2). The effect of chromotropic acid purity on this reaction has been more thoroughly investigated recently (13).

The A.S.T.A. procedure for development of the color was followed with the exception that the 25-ml. volumetric flasks containing 1 ml. of either the sample, the blank, or the piperine solutions, and 0.5 ml. of the aqueous chromotropic acid solution were cooled in an ice-water-salt bath.

SPECTROPHOTOMETRIC ULTRAVIOLET METHOD. A portion of the ethanolic extract obtained from the above-mentioned extraction of 0.8 gram of ground pepper with 250 ml. of ethanol was filtered before use and kept in brown glass bottles. Depending on piperine content, preferably 3 or sometimes 2 ml. of this filtrate were transferred into 100-ml. volumetric flasks, and the flasks were immediately filled up to volume with ethanol. The ultraviolet absorption at the maximum, 343 $m\mu$, was read against a solvent blank in a Beckman DU Spectrophotometer. The apparent piperine content was calculated from the standard curve which has the following equation:

$$\text{Apparent piperine (mg./1000 ml.)} = 0.75 \times (\text{absorbance at } 343 \text{ m}\mu)$$

This holds for concentrations giving absorbances of 0.1 to 0.85.

In addition to reading the ultraviolet absorbance of piperine at 343 $m\mu$, the absorbance of piperettyne was measured at 364 $m\mu$ (14). The true piperine and piperettyne contents were calculated from the molecular absorbances of the two compounds by a two-component equation (7). For a mixture of piperine and piperettyne solutions, the absorbance at a given wavelength is found to be additive. In such mixtures, the concentrations of these two components can be obtained from the following equations:

$$A_{343} = c_{\text{piperine}} \times \frac{A_{343} \text{ of std. piperine soln.}}{c_{\text{std. piperine soln.}}} + c_{\text{piperettyne}} \times \frac{A_{343} \text{ of std. piperettyne soln.}}{c_{\text{std. piperettyne soln.}}} \quad (1)$$

$$A_{364} = c_{\text{piperine}} \times \frac{A_{364} \text{ of std. piperine soln.}}{c_{\text{std. piperine soln.}}} + c_{\text{piperettyne}} \times \frac{A_{364} \text{ of std. piperettyne soln.}}{c_{\text{std. piperettyne soln.}}} \quad (2)$$

where A is the absorbance at the wavelength indicated in $m\mu$ in the subscript, and c is the concentration of the indicated substance in grams per liter. The ratio of absorbance to concentration is constant because the solutions obey Beer's law over the range of concentrations studied (0 to 8 mg. per liter). The following constants, replacing these ratios

in Equations 1 and 2, have been determined from measurements on a number of standard solutions of piperine and piperettyne, thus:

$$A_{343} = (c_{\text{piperine}}) 0.1323 + (c_{\text{piperettyne}}) 0.1186$$

$$A_{364} = (c_{\text{piperine}}) 0.0812 + (c_{\text{piperettyne}}) 0.5131$$

The final formulas after incorporating dilution and percentage factors for 3 or 2 ml. of filtrate were:

$$\% \text{ Piperine} = (14.40 A_{343} - 11.16 A_{364}) \times 1.042 \text{ if 3 ml. is used}$$

$$\text{or} \quad \times 1.56 \text{ if 2 ml. is used}$$

$$\% \text{ Piperettyne} = (12.45 A_{364} - 7.641 A_{343}) \times 1.042 \text{ if 3 ml. is used}$$

$$\text{or} \quad \times 1.56 \text{ if 2 ml. is used}$$

COLUMN CHROMATOGRAPHY. The glass column apparatus (12) was filled with redistilled benzene and then packed with 50 grams of activated Florisil suspended in benzene. During packing, the Florisil-benzene mixture was stirred continuously to prevent formation of air pockets and segregation of Florisil particles according to particle size. The tube was filled up to 1 cm. below the reservoir outlet. A glass-wool plug on top of the column kept the absorbent in place. A sample of 2.2 grams of the residual oleoresin from the ethanolic extraction of white pepper was dissolved in 20 ml. of benzene. This benzene solution containing the oleoresin was introduced gradually at the top of the column

through a dropping funnel at the rate of one drop per second.

As soon as all of the oleoresin was on the Florisil, the solvent reservoir (12) was put on top of the column and filled successively with 200 ml. of benzene-pyridine (90:10), then with 150 ml. of benzene-pyridine (1:1), and finally with 150 ml. of pyridine alone. On development, several bands occurred ranging from a wide brown band on top to various yellow-shaded bands underneath. Ten fractions of 50 ml. each were collected.

After the eluting solvents had been completely evaporated, the residues were dissolved in either ethanol, chloroform, or carbon disulfide, and ultraviolet and infrared spectra of all these fractions were taken. In addition, the colorimetric determination using chromotropic acid was carried out on equal aliquots of some of these fractions.

SYNTHESIS OF PIPERINE ANALOGS. Piperonylic acid piperidide and meth-

ylene caffeic acid piperidide were synthesized. Their formulas are given in Figure 1. The piperonylic acid piperidide was prepared from piperonylic acid by the procedure of Staudinger and Schneider (15). Methylene caffeic acid piperidide was obtained from piperonal using Doebner's procedure (3). Ultraviolet spectra of these piperidides at convenient concentrations are given in Figure 2.

PAPER CHROMATOGRAPHY. For identification purposes, reversed-phase ascending paper chromatography was used to separate piperine, piperettine, other pepper constituents, and the synthesized analogs mentioned above. An 11- × 11-inch 3MM Whatman paper was impregnated with 10% Silicone 550 in acetone. When the paper was dry, 5 μ l. of a 1% ethanolic solution of each individual compound or a composite solution of these compounds was spotted 3 cm. from the lower edge of the paper.

The papers were run in a solvent consisting of *n*-propanol-water-diethylamine (1:8:1) (5). A good separation of the compounds was achieved within 8 hours. The spots were identified in ultraviolet light (366 m μ) and by spraying with the Lüdy-Tenger reagent (10) or with diazotized sulphanilic acid. This paper chromatographic system was applied to ethanolic extracts of black and white pepper and various column fractions.

THIN-LAYER CHROMATOGRAPHY. The same impregnation and the same solvent were used as in the paper chromatographic procedure above. Glass plates 8 × 8 inches were layered with Silica Gel G (layer thickness approximately 275 microns). In addition to identification by ultraviolet light and spray reagents used on paper, some of the thin-layer chromatograms were also sprayed with 25% chromotropic acid in distilled water and then with concentrated sul-

Table I. Apparent Percentage Piperine Found in *Piper nigrum* L. by Colorimetric and Ultraviolet Procedures

Sample Description	Ultraviolet Spectrophotometric Procedure	Colorimetric Method Using Conversion Constant ^a from:	
		A.S.T.A.	These laboratories ^b
BLACK PEPPERS			
Indian			
Malabar 706	5.67	5.90	6.94
	5.63	5.85	6.88
733	5.59		
	5.49	7.15	8.48
Ceylon			
Matale 714	5.10	5.89	6.98
Indonesian			
Sumatra 742	5.52		
	5.65	6.34	7.46
Lamong 644	5.21	5.73	6.75
	5.29	5.76	6.78
	5.00	6.05	7.12
643	4.92	6.37	7.50
695	5.65	6.08	7.15
	5.52	6.26	7.36
688	6.44	7.14	8.45
Sarawak 689	5.70	6.55	7.76
Unidentified 694	5.86	6.16	7.25
	5.57	6.27	7.38
722	5.92	5.98	7.04
	5.99	6.53	7.68
		6.14	7.23
WHITE PEPPERS			
Indian 705	3.86	4.15	4.88
	3.72	4.66	5.49
	3.70		
Indonesian			
Muntok 642	5.02	5.54	6.51
	5.13	5.55	6.53
698	4.71	5.35	6.30
	4.73	5.74	6.75
	4.63		
Unidentified 721	5.03	6.52	7.68
	5.11	7.00	8.23
		5.78	6.80
Ceylon			
Kandy 715	8.54 ± 0.11 ^c	9.79 ± 0.39	11.59 ± 0.46

^a The conversion constant permits the conversion of absorbance readings at 580 m μ directly to percent piperine.

^b Obtained using pure piperine solution under the same conditions as used in the determinations of the piperine in the peppers.

^c Average of 10 determinations with indicated std. dev.

Table II. Ultraviolet Spectrophotometric Analysis of Peppers for Piperine and Piperettine

Sample Description	Piperine ^a , %	Piperettine ^a , %	Ratio of Piperine to Piperettine
BLACK PEPPERS			
Indian			
Malabar	4.25	0.46	9.1
	4.02	0.77	5.2
	4.36	0.67	6.5
	4.03	0.72	5.6
	4.39	0.71	6.2
6 samples, from shipper A	3.98-4.54	0.66-0.81	5.4-6.9
4 samples, from shipper B	4.16-4.25	0.59-0.82	5.1-7.1
immature "Tellicherry"	4.27	1.08	4.0
Balamcotta var.	3.12	0.43	7.2
Kalluvalli var.	4.05	0.50	8.0
Unspecified	4.48	0.23	19.4
Ceylon	3.86	0.77	5.0
	7.44	1.56	4.8
	4.45	0.74	6.1
Indonesian			
Sumatra	5.15	0.44	11.7
Java	5.09	0.37	13.7
Lamong	4.99	0.33	15.3
	6.14	0.41	15.1
	5.17	0.39	13.4
	4.76	0.35	13.5
Sarawak	5.52	0.41	13.3
	5.13	0.32	16.0
	5.55	0.44	12.7
	5.35	0.38	14.3
Jamaica	3.72	0.57	6.6
Brazil	3.66	0.36	10.3
WHITE PEPPERS			
Ceylon			
Kandy	7.73	0.86	9.0
Indonesian			
Sumatra	4.02	0.46	8.9
Java	4.05	0.54	7.5
Muntok	4.45	0.65	6.9
	4.30	0.49	8.7
	4.29	0.41	10.4
Sarawak	4.33	0.49	8.8
	4.01	0.60	6.7
	4.58	0.48	9.5
	4.78	0.47	10.2

^a All results are for different individual samples, with no duplication.

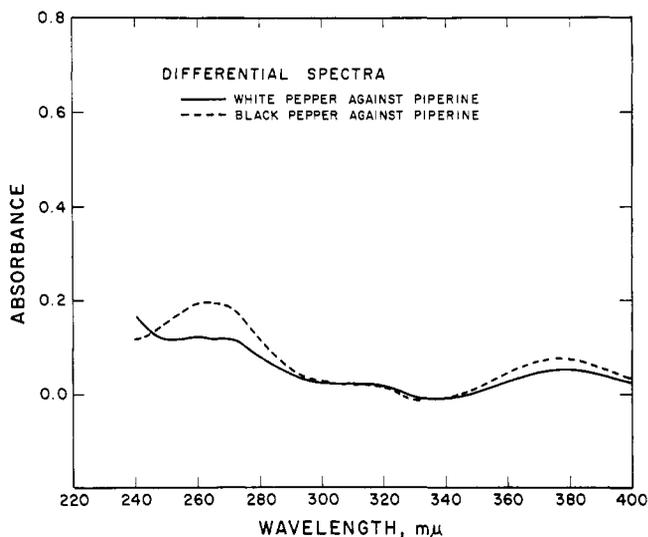


Figure 3. Differential ultraviolet spectra of white and black pepper against piperine at a concentration to give the same absorbance at 343 m μ

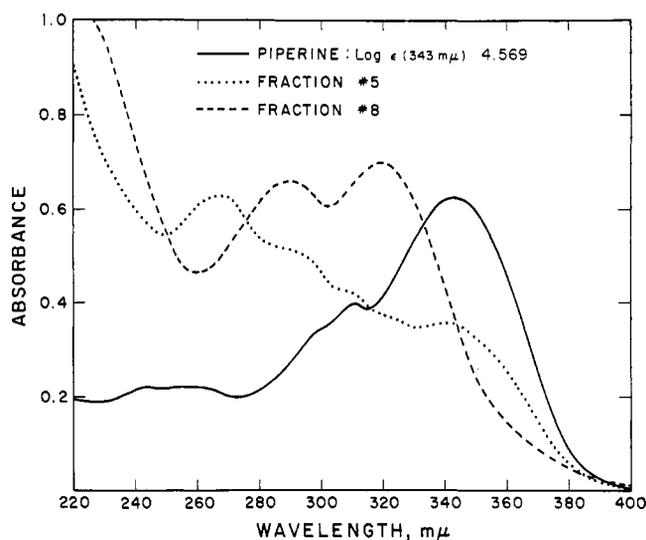


Figure 4. Qualitative absorption spectra of fraction 5, fraction 8, and piperine

furic acid to develop purple spots from methylenedioxy-containing compounds.

Results and Discussion

Several variations of the A.S.T.A. procedure were tried in order to find the cause for the irregular results obtained even when pure piperine was used. Results from these determinations using the purified piperine were compared by calculating the constant used in converting absorbance readings at 580 m μ to percent piperine. Cooling in an ice-water-salt bath was introduced to avoid excessive heating upon addition of the concentrated sulfuric acid. This improved the reproducibility somewhat. Time intervals between heating and cooling steps were also kept constant. In accordance with the report of Bricker and Johnson (2), increased hydrolysis times did not have any marked effect on the piperine values. The use of 25% chromotropic acid solution appeared to give results for a constant more in accord with that reported in the methods book of the A.S.T.A. (17). Two batches of chromotropic acid produced different results for the conversion constant. However, the pepper results reported in Table I were obtained using the lower, recommended concentration of chromotropic acid. Diethoxymethane (formaldehyde diethylacetate) when used as an additional standard for the chromotropic acid procedure required a longer hydrolysis time than piperine for complete release of formaldehyde and therefore was not satisfactory. None of these suggested improvements for the A.S.T.A. procedure gave chromotropic values in accord with the results obtained by the ultraviolet spectrophotometric method.

The results presented in Table I show clearly that the apparent piperine values obtained with the A.S.T.A. colorimetric procedure differ considerably from those obtained by the ultraviolet method. Precision of results was within $\pm 3\%$ by the ultraviolet method with one exception (694), whereas by the chromotropic acid procedure many results varied by 10 and even 20%. The standard deviation of both methods given in Table I was calculated from 10 piperine determinations on the same pepper. Since the pepper extract was used for the analysis without purification, closely related analogs of piperine which also have a methylenedioxy grouping, such as piperettine (14) or methylene caffeic acid piperidide, may have reacted with chromotropic acid, thereby creating higher "piperine" values. For this reason, the pepper extract was investigated further to find out whether it contained components other than piperine which could react with the chromotropic acid reagent.

Differential ultraviolet spectra measured by comparing white or black pepper extracts against a pure piperine standard of equal absorbance at the 343 m μ maximum (6) are given in Figure 3. These spectra indicated that substances with an ultraviolet absorption maximum around 260 to 270 m μ are present in the pepper. However, the difference spectra did not correspond to the spectra of either the synthesized methylene caffeic acid piperidide or the piperonylic acid piperidide. On the other hand, there was a peak corresponding to piperettine, a reported constituent of black pepper (14).

Based on the additive relationship between the piperine and piperettine spectra given in Figure 2, the analyses

for piperine and piperettine contents were calculated and reported in Table II. Calculations are also given of the ratios of the two biochemically related constituents according to the geographical area of growth. In general, Indonesian black peppers appear to have a ratio of piperine to piperettine greater than 10, while with Indian black peppers the ratio is less than 10. White Indonesian peppers have a lower ratio than the black peppers, but this may be due to any one of the factors differentiating white and black peppers, such as the degree of ripeness or the removal of the outer hull.

In the search for other identifiable components, 93% of the pepper extract put on the column was recovered as fractions eluted from the Florisil with solvents of increasing polarity. The major fraction (85% of the recovered material) consisted of piperine and piperettine. The only other fractions of note were fractions 5 (2%) and fraction 8 (3%). The qualitative ultraviolet spectra of the later fractions differ from the original piperine spectrum in that the maximum has been shifted to a shorter wavelength as can be seen from Figure 4.

All the fractions gave a positive Labat test (8) and a positive reaction with chromotropic acid, thereby suggesting the possibility that all fractions contained a component with a methylenedioxy grouping. When it was assumed that all the absorption of these fractions at 343 m μ was due to piperine, then the formaldehyde from the methylenedioxy group, as determined colorimetrically, corresponded to more than the quantity expected from the amount of piperine indicated by the ultraviolet method. This again suggested the presence of methylenedioxy groups in these fractions.

Table III. Paper Chromatographic Behavior of Pepper Extract Constituents and Synthesized Analogs of Piperine

Spot No.	Identification	Approx. R_f	Ultraviolet Light, 366 $m\mu$	Reaction when Sprayed with:	
				Lüdy-Tenger	Diazotized sulphanic acid
1	Piperettine	0.1	Yellow spot	No reaction	No reaction
2	Piperine	0.24	Dark spot	Weak orange spot	No reaction
3	Unknown	0.41	Dark spot	No reaction	No reaction
4	Piperonylic acid piperidide	0.68	No reaction	Strong orange spot	No reaction
5	Unknown	0.9	Strong light blue fluorescence	No reaction	Blue spot
6	Methylene caffeic acid piperidide	0.9	Weak dark blue spot	No reaction	No reaction

On the basis of the shift of the ultraviolet maximum to a lower wavelength in the later column fractions and the apparent methylenedioxy content, it was anticipated that these fractions might have as their main constituents compounds such as piperonylic acid piperidide or methylene caffeic acid piperidide. The spectra of these compounds are given in Figure 2.

The paper chromatographic procedure showed four spots in ethanolic extracts of white and black pepper as indicated in Table III. None of the spots was identical to piperonylic acid piperidide nor methylene caffeic acid piperidide in spite of the similarity of the ultraviolet spectrum and the R_f value of methylene caffeic acid piperidide and fraction 8 illustrated in Figures 2 and 4 and Table III, respectively. Spot No. 5, the main component in fraction 8, at the R_f of methylene caffeic acid piperidide has a strong blue fluorescence in ultraviolet light and reacts with diazotized sulphanic acid. Methylene caffeic acid piperidide, on the contrary, shows a

weak, dark blue color under the ultraviolet and does not react with diazotized sulphanic acid. Although thin-layer chromatography resolves fraction 8 into several additional bands the evidence did not indicate that methylene caffeic acid piperidide was one of the components of this fraction.

A spot with the same R_f as piperettine was present in all peppers. Piperine and piperettine react with chromotropic acid and concentrated sulfuric acid on thin-layer chromatograms, whereas no spray reagent could be found for spot No. 3. Spot No. 3 is the main component of the fraction 5 obtained by column chromatography. Black and white pepper can also be distinguished by thin-layer chromatography and examination under ultraviolet light since some bands which are not present in any of the white peppers occur in black pepper.

Acknowledgment

The authors acknowledge the kind offices of Leo Levi in obtaining the sam-

ples and the work of W. Skakum in the preparation of the samples. Thanks are also due to John McLean for placing at their disposal a sample of pure piperettine. The authors are also grateful to R. A. Chapman for his encouragement of this study.

Literature Cited

- (1) Assoc. Offic. Agr. Chemists, Washington, D. C., "Methods of Analysis," 8th ed., p. 515, 1955.
- (2) Bricker, C. E., Johnson, H. R., *Ind. Eng. Chem., Anal. Ed.* **17**, 400 (1945).
- (3) Doebner, O., *Ber.* **35**, 2137 (1902).
- (4) Fagen, H. J., Kolen, E. P., Hussong, R. V., *J. Agr. Food Chem.* **3**, 860 (1955).
- (5) Genest, K., Farmilo, C. G., *J. Chromatog.* **6**, 343 (1961).
- (6) Hiskey, C. F., *Anal. Chem.* **33**, 927, (1961).
- (7) Jones, M., Thatcher, R. L., *Ibid.*, **23**, 957 (1951).
- (8) Labat, A., *Bull. Soc. Chim. France* [4] **5**, 745 (1909).
- (9) Lee, L. A., *Anal. Chem.* **28**, 1621 (1956).
- (10) Lüdy-Tenger, F., *Mikrochemie ver. Mikrochim. Acta* **38**, 460 (1952).
- (11) "Official Analytical Methods," American Spice Trade Association, Method 13, p. 32, 1960.
- (12) Sahasrabudhe, M. R., Chapman, D. G., *J. Am. Oil Chemists' Soc.* **38**, 88 (1961).
- (13) Sawicki, E., Hauser, T. R., McPherson, S., *Anal. Chem.* **34**, 1460 (1962).
- (14) Spring, F. S., Stark, J., *J. Chem. Soc.* **1950**, 1177.
- (15) Staudinger, H., Schneider, H., *Ber.* **56**, 699 (1923).
- (16) Tausig, F., Suzuki, J. I., Morse, R. E., *Food Technol.* **10**, 151 (1956).

Received for review July 30, 1962. Accepted November 29, 1962. Presented at the 45th Canadian Chemical Conference of the Chemical Institute of Canada, May 27-30, 1962, Edmonton, Alberta.

RICE QUALITY MEASUREMENT

Organic Acids of Rice and Some Other Cereal Seeds

ANALYSIS of the organic acid mixtures in rice of different varieties and conditions was prompted by the belief that such metabolically active substances would act as key compounds for indicating other compositional varia-

¹ Present address: Wilkens Instrument and Research, Inc., P. O. Box 313, Walnut Creek, Calif.

² Deceased

tion. Though present only as minor constituents, the organic acids are more susceptible to measurement of small changes than the major constituents, starch and protein. Hence, they might serve as sensitive indicators of quality changes.

Organic acids of plants (aside from acids in fats) are comprised chiefly of nonvolatile, nonnitrogenous aliphatic

acids commonly called plant acids (2). Their occurrence is widespread, and they play a central role in cellular metabolism. Reports on their presence, quantities, and changes have generally been for actively metabolizing tissue and usually for noncereal plants (2, 3). These acids are often accompanied by small amounts of aromatic acids which may act as growth regulators or germina-

DAVID F. HOUSTON, BEVERLY E. HILL,¹
VILMA H. GARRETT, and E. B. KESTER²
Western Regional Research
Laboratory, Albany, Calif.