Reprinted from The Flavour Industry, 1, 173-175(1970)

Colorimetric Method of Determination of Pungent Constituents of Pepper

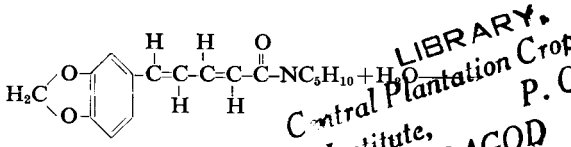
*M. L. SHANKARANARAYANA, S. NAGALAKSHMI and C. P. NATARAJAN**

SUMMARY

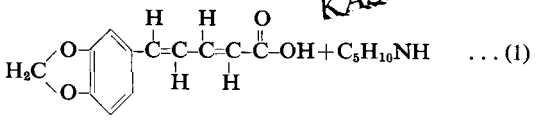
Piperine is an important predominating pungent constituent in pepper. Pepper also contains small quantities of chavicine and piperettine which possess sharp biting and pungency. A survey of the existing methods showed that the ultraviolet spectrophotometric method which is widely used is specific for piperine only and not for the other pungent constituents. In the method described here, it is possible to determine the total pungency due to piperine, chavicine and piperettine. The method consists of hydrolyzing the piperidine containing pungent constituents to liberate piperidine which is reacted with carbon disulphide to form piperidinium pentamethylene dithiocarbamate. Piperidinium pentamethylene dithiocarbamate reacts with copper sulphate to give a yellow colour which has an absorption maximum at 435mμ. One mole of piperine on hydrolysis gives one mole of piperidine. The cupric complex obeys Beer's law between a concentration of 1 to 14μg/ml of piperidine. The method is applicable to determine the trace quantities of the total pungent constituents present in pepper and also in the oleoresin of pepper.

Pepper is one of the most important of spices used for both flavour and aroma. Piperine is known to be the predominating constituent responsible for the sharp biting and pungency. The quality of the pepper and also of the oleoresin of pepper is dependent on the amount of piperine present in them. The value of pepper is, therefore, dependent on its piperine content. Hence, methods for the estimation of piperine are becoming more important. Recently, Graham[1] and Labruyere[2] have reviewed the available methods and pointed out their merits and demerits. Among the various methods the spectrophotometric method[3, 4, 5] is very specific for piperine and reliable under controlled conditions. However, it is known that there are other constituents such as chavicine[6] and piperettine[7] which are also responsible for the pungency. The spectrophotometric method fails to measure the presence of these and it has been found that in dilute solutions the piperine which has trans-trans configuration slowly transforms to its isomeric form chavicine[8] (cis-cis configuration). The present method is based on the alkaline hydrolysis of piperidine containing compounds to liberate quantitative piperidine[2]. The piperidine is reacted with carbondisulphide to get piperidinium pentamethylenedithiocarbamate[9] which gives a yellow colour with a copper salt forming copper piperidine pentamethylene dithiocarbamate[10]. The various above reactions using a typical example, piperine are represented below: The yellow colour of the complex has an absorption maximum at 435mμ. In fact, the dialkyldithiocarbamates[11] are an important group of reagents for the detection and determination of traces of copper.

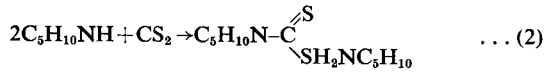
*Central Food Technological Research Institute, Mysore, India.



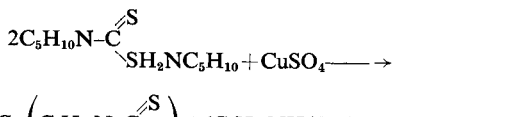
Piperine



Piperic acid Piperidine



Piperidinium pentamethylene dithiocarbamate



Cupric piperidinium pentamethylene dithiocarbamate

EXPERIMENTAL

Reagents, materials and apparatus

Piperidine: (Fluka, analytical grade) was distilled before use. A stock solution was prepared by dissolving 80mg of piperidine in acetone (AR) and diluting to 100ml in a volumetric flask. A solution containing 100μg/ml was prepared by suitable dilution with the acetone.

Carbon disulphide: (Southern Organic Chemical Industries, Madras).

This was distilled and 3g of it was dissolved in 100ml acetone.

Copper sulphate solution: A 0.1% aqueous solution (w/v) of copper sulphate pentahydrate (AR quality) was used.

2N Potassium hydroxide in propylene glycol: 112g of potassium hydroxide (AR quality) was dissolved in 80g of water and diluted to 1 litre with propylene glycol (BDH of B.P. 186–188°C).

Black pepper: A sample available in the local market was finely ground, passed through 30 mesh and used.

Oleoresin of black pepper: This was prepared by extracting the ground pepper powder repeatedly with diethylene chloride, and concentrating it, until it was free from the solvent.

Piperine: This was isolated from oleoresin of black pepper first by washing with pet. ether and then by repeatedly

extracting it with hot ethanol. It was later purified by recrystallization from ethanol to get pale yellow colour crystals with m.p. 129–130°C. It showed an absorption maximum at 345mμ.

Spectrometer: Absorption measurements were made with a Spectronic-20 colorimeter/Spectrophotometer, Bausch and Lomb and Beckman DU Spectrophotometer.

PROCEDURE

Perparation of a calibrated chart for piperidine

To 1ml of the piperidine solution (containing varying amounts of piperidine[1] to 14μg) taken in a test tube maintained at 0°C was added 7ml of acetone. To this 1ml of previously cooled carbon disulphide reagent was added, shaken well and the tube further dipped in ice for 5min for the completion of the reaction (Eqn. 2). Then 1ml of the copper sulphate solution was added and a light yellow coloured solution was obtained (Eqn. 3). The volume of the solution in the test tube was made up to 20ml with acetone. The absorbance of the solution was measured at 435mμ using a reagent blank. The results of analysis are included in Table I.

Table I. Results of Analysis of piperidine

	Piperidine	
	Taken $\mu g/ml$	*Found* $\mu g/ml$
1	1.0	0.095
2	2.5	2.450
3	4.3	4.250
4	6.1	6.050
5	8.4	8.350
6	11.6	11.550
7	12.5	12.450
8	13.8	13.750

Estimation of pure piperine

A known weight of piperine (about 0.1g) was transferred to a 250ml round bottomed flask. About 50ml of 2N KOH in propylene glycol were added to this and refluxed using a Liebig's water condenser for 2h on a sand bath maintaining the temperature around 140°C. The mixture was cooled to the room temp. About 100ml of water and a few drops of antifoam reagent (silicone compound) were added to the flask. The refluxing condenser was removed and the flask was connected to a distillation set. The mixture was distilled for nearly an hour and the distillate was absorbed in a conical flask containing about 30ml acetone. The distillation was stopped when the distillate was neutral to indicator paper (*i.e.*, pH 7). The distillate was divided into two equal portions. The first portion (say about 50ml) was titrated with 0.01 N hydrochloric acid using a few drops of mixed indicator of bromo-cresol green and methyl red as described by Labruyere[2]. The second portion (say about 50ml) was diluted with acetone (to a volume of about 200ml) to get 20 to 100μg/ml of piperidine based on theoretical calculation. The piperidine was estimated as described above. The amount of piperidine was calculated from a standard graph and the purity of the sample computed. The purity of the piperine was also determined by the ultraviolet spectrophotometric method[3]. The results of analysis are given in Table II.

Determination of pungent constituents in black pepper and oleoresin of pepper:

Black pepper: 1g of ground black pepper was extracted with about 100ml acetone in a soxhlet apparatus for 20h. The solvent acetone was removed by heating the flask on a hot water bath (temp. 65°C) to obtain a green syrupy liquid of

Table II. Analysis of piperine

	Weight piperine taken in g.	*Hours of refluxion*	*Piperine found in %*		
			By the titrimetric method	*By the colorimetric method*	*By the ultraviolet spectrophotometric method*
1	0 1	2	99.00	99.5	99.8
2	0.1	2	99.72	100.0	99.5
3	0.1	2	98.33	99.33	100.0
4	0.1	2	98.40	99.00	100.2

Table III. Analysis of black pepper and oleoresin of black pepper

Sample	*Weight taken in g*	*Pungent constituents found in % (expressed as Piperine)*	
		Titrimetric method	*Colorimetric method*
Black Pepper			
1	1.0	5.32	5.34
2	1.0	5.46	5.50
3	1.0	5.28	5.32
4	1.0	5.38	5.42
Oleoresin of black pepper			
1	0.2	47.30	47.35
2	0.2	47.60	46.54
3	0.2	47.32	47.54
4	0.2	46.70	47.50

Table IV. Effect of hours of extraction using acetone in the determination of pungent constituents in black pepper

Weight of black pepper taken g		*No. of hours of extraction with acetone*	*Pungent constituents in % (expressed as piperine)*	
			By titrimetric method	*By colorimetric method*
1	1.0	4	4.28 4.21 4.20	4.30 4.36 4.22
2	1.0	8	5.01 5.03 5.04	5.03 5.05 5.06
3	1.0	12	5.10 5.20 5.10	5.03 5.05 5.20
4	1.0	16	5.30 5.29 5.35	5.32 5.40 5.33
5	1.0	20	5.40 5.41 5.45	5.42 5.40 5.50
6	1.0	24	5,42 5.40 5.40	5.50 5.41 5.38

oleoresin of pepper. This was hydrolyzed with propylene glycol containing KOH and the piperidine containing pungent constituents (expressed as piperine) determined as described above by both the titrimetric and the colorimetric methods. The results of analysis are given in Table III. The Table IV includes data regarding the hours of extraction of piperine from the black pepper.

Oleoresin of pepper: An accurately weighed sample of about 0.2g of well homogenized oleoresin of pepper was transferred

to a round bottomed flask. It was refluxed for 2h using the propylene glycol containing KOH and the piperidine containing pungent constituents determined by both the titrimetric method and the colorimetric method described here. The results of analysis are included in Table III. From the experimental value of the weight of piperidine found, the pungent constituents expressed as the percentage of the piperine was computed using the following equation:

$$\text{Piperine in } \% = \frac{\text{Weight of piperidine found in g} \times 3.353 \times 100}{\text{Weight of oleoresin or black pepper in g}}$$

One mole of piperine (mol. wt. 285) gives on hydrolysis one mole of piperidine (mol. wt. 85).

Discussion: It can be seen from Table I that the accuracy obtained is quite satisfactory. The copper piperidinium pentamethylene dithiocarbamate was found to obey Beer's law between a concentration range of 1 to 14μg/ml of piperidine, but deviated beyond this concentration.

The data in Table IV show the studies carried out to find out the minimum number of hours necessary for the complete extraction of the biting pungent constituents from the black ground pepper. It was found that 20h were essential for the complete extraction.

The absorbance readings of the complex were measured after every half an hour for a period of 4h and the results showed no change in the colour. This shows that the colour of the complex did not fade in presence of light contrary to the reported data in similar studies[10, 11]. This can be probably explained by the fact that in the present study there is a large excess of the cupric sulphate and, therefore, there is no chance of the reduction of the cupric dithiocarbamate to cuprous compound.

The Table I shows a fairly good agreement between the titrimetric and the colorimetric method. For the hydrolysis of the piperine both diethylene glycol and propylene glycol containing KOH were used. The propylene glycol was preferred because the reagent was stable for a number of days. The purity of the piperine was found to be 100% pure by the ultraviolet spectrophotometric method. This further shows that the colorimetric method is comparable with the ultraviolet spectrophotometric method. However, the comparison between the u.v. and the colorimetric methods is valid only for the pure piperine and not when it is determined in the oleoresin or black pepper which contains other constituents giving piperidine on hydrolysis.

The results of Table III show the colorimetric method is also applicable to the black pepper and the oleoresin of pepper. The ultraviolet spectrophotometric method is specific for piperine and not for other pungent constituents such as chavicine and piperettine ($C^{29}H^{21}O^3N$) which are present in the black pepper. While in the present method the amidic bond which is present in piperine, chavicine and piperettine releases on hydrolysis the piperidine which is colorimetrically determined. It has been pointed out that it is the amidic bond which is responsible for the pungency. In a sample of oleoresin of pepper containing the 1-cinnamoyl piperidine[12] (a synthetic pungent compound) which is used as an adulterant, the ultraviolet spectrophotometric method is able to distinguish and give the piperine content. Whereas the present method will give the total pungent constituents present in the sample. Therefore, the ultraviolet spectrophotometric method is a useful complimentary method in determining the piperine content and also the other pungent constituents, if present in a sample of the material.

The advantage of the present method over the titrimetric method of Labruyere is that the method is applicable to determine the trace quantities of the biting constituents. However, the hydrolysis method itself suffers from the one disadvantage that the material should be free from amides which do not possess the pungent factor.

Acknowledgement

The authors wish to thank Dr. H. A. B. Parpia, Director of the Institute for his keen interest in the work.

REFERENCES

[1]H. D. Graham, *J. Food Sci.*, **30,** (1965), p. 664.
[2]B. Labruyere, *J, Agr. Food Chem.*, **14,** (1966), p. 469.
[3]H. J. Fagen, E. P. Kolen and R. V. Hussong, *J. Agr. Food Chem.*, **3,** (1955), p. 860.
[4]T. N. Ramachandra Rao, C. T. Dwarakanath and D. S. Johar, *J. Proc. Inst. Chem. (Ind.)*, **32,** (1960), p. 125.
[5]C. Genest, D. M. Smith and D. G. Chapman, *J. Agr. Food Chem.*, **11,** (1963), p. 508.
[6]E. H. Rodd, *Chemistry of Carbon Compounds, Vol. IV. Part C. Heterocyclic Compounds*, Elserier Publg. Co. Amsterdam, (1960), p. 1803.
[7]F. S. Spring and J. Stark, *J. Chem. Soc.*, 1177 (1950).
[8]F. Taussig, J. I. Suzuki and R. F. Morse, *Food Technology*, **10,** (1956), p. 151.
[9]M. L. Shankaranarayana and C. C. Patel, *Anal. Chem.*, **33,** (1961), p. 1398.
[10]C. G. Ramachandran Nair, V. R. S. Rao and A. R. Vasudeva Murthy, *Mikrochim. Acta*, (1961), p. 741.
[11]T. A .Callan and J. A. R. Henderson, *Analyst*, **54,** (1929), p. 650.
[12]E. L. Wick and S. E. Cairncross, *Food Technology*, **10,** (1956), p. 423.

REPRINTED FROM THE FLAVOUR INDUSTRY, VOLUME 1, NUMBER 3, MARCH 1970, PAGE 173.

ERRATA:

PAGE 1. EQUATION 3

$(C_5H_{10}NH_2)H_2SO_4$ should be $(C_5H_{10}NH)_2 \cdot H_2SO_4$

PAGE 3. piperettine $C^{29}H^{21}O^3N$ should be $C_{19}H_{21}O_3N$

Mixing Equipment

We have received a brochure from Millroom Accessories & Chemicals Limited, Protector Works, 547 Liverpool Road, Irlam, Manchester, describing the Biotomix. This is a twin disperser and mixer which has the following features: mobility; high-shear rate; stability; all-power input is used in the mix; low price; interchangeable blades; two-speed motor. This mixer-disperser can be used for dispersions and emulsions.

New Activities for LKB Analytical Instruments Division

LKB Instruments Ltd. have been active in pioneering the use of combination gas chromatography—mass spectrometry in a variety of areas—organic biochemistry, natural products, clinical chemistry, food analysis, geo-chemistry and many others.

Their activities will now be extended to other areas of mass spectrometry under the terms of an agreement with Electronic Associates Incorporated, Scientific Instrument Division, Palo Alto, California, USA to market in the UK and Eire the Quad line of quadrupole mass spectrometers. The conclusion of this agreement completes the exclusive coverage of these instruments by LKB companies throughout Europe, including Russia.

Quadrupole mass spectrometers, though developed from discoveries within the last fifteen years, are already in use in many 'typical' fields of mass spectrometry. Their very short scan times and complete ease of operation have brought them into service in applications of a special nature. Applications range from petroleum chemistry, process control, air pollution and isotope analysis to respiratory gas analysis, medicine, education, kinetic and mechanism studies.

Further information is available from Mr. J. Maxwell Jones, Product Manager, Analytical Instruments Division, LKB Instruments Ltd., LKB House, 232 Addington Road, South Croydon, Surrey, CR2 8YD, Tel. No. 01-657-0286.

Techniques in Gas Chromatography

In *The Analyst* **95**, No. 1126 (January 1970), pp. 1 to 15, there is a paper, 'Techniques in Gas Chromatography, Part III, Choice of Detectors', a review by T. A. Gough and E. A. Walker. In this review the advantages and disadvantages of a large represented selection of detectors are set out.

High pressure Pump

Metering Pumps Ltd. have designed and manufactured a new 150hp diesel driven four-wheeled trailer high pressure jetting unit, which is capable of producing pressures of 10,000psi at a water flow of 16gal/min, or 3,500psi at 47gal/min.

The first unit has been supplied to one of the principal UK contracting companies for use in chemical and petroleum undertakings, and will also be used in the marine industry.

Exhibition of American Biochemical Test and Control Equipment

The great progress made by biochemistry in the last twenty years has necessitated the development of increasingly more advanced and sophisticated apparatus. A wide variety of new developments will be on show at an exhibition of American biochemical test and control equipment at the US Trade Center, 57 St. James's Street, London SW1 (February 16th-20th).

The exhibition will feature many types of spectrophotometers, including the first complete system available for studying rapid reactions in the ten microsecond to fifty second range. This instrument is also suitable for combined stopped-flow and temperature-jump studies in the u.v. visible range. A temperature-jump accessory is available for studies involving even faster reactions.

Chromatography and electrophoresis will be well represented. Of particular interest is a system that allows all forms of liquid chromatography to be used in the separation and detection of thermally labile or high molecular weight samples: the techniques available include absorption, partition, gel permeation, ion-exchange and supercritical fluid chromatography. A variety of ancillary equipment will also be on show, including a radio-chromatogram scanner, monitoring equipment for chromatography of u.v. absorbing compounds, fraction collectors and sample-changers.

The Pungent Principles of Ginger

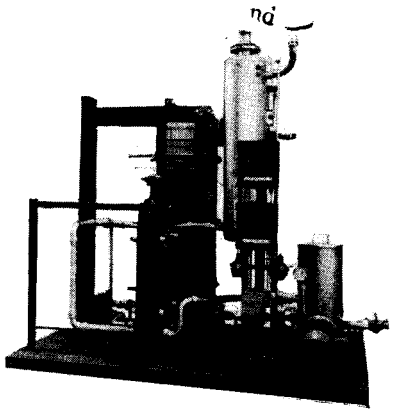
We have received a reprint of a paper from *Food Technology in Australia*, **21**, No. 11 (November 1969), pp. 570-5, with the title 'The Pungent Principles of Ginger and their Importance in Certain Ginger Products', by Dr. D. W. Connell. In this paper the author investigated the pungent principles of Ginger and found it to be shugaol and not the expected gingerol. The structure of gingerol was finally elucidated 100 years after it was first isolated by Thresh. The reactions of gingerol are discussed and the changes that take place during storage. A new pungent principle was discovered. The author stresses the importance of non-volatile taste substances in flavours.

Oxidation Spoilage

Naarden News, **21**, No. 211 (January 1970), has an article, 'Spoilage Through Oxydation,' in which the causes are discussed and hints given on how to prevent it.

Reprint Received

We have received a copy of a reprint from the *Australian Journal of Chemistry* 1966, **19**, 283-8 with the title 'Terpenoid Chemistry XI (-)-ß-sesquiphellandrene' by D. W. Connell and M. D. Sutherland. This sesquiterpene was isolated from oil of ginger and shown to be (—)-ß-Sesquiphellandrene is (1'R,6S)-2-methyl-6-(4'-methylene-cyclohex-2'-enyl)hept-2-ene.



APV Pure Steam Generating Plant

Where steam of absolute purity is required for sterilizing or other purposes, APV can provide a packaged pure steam generating plant. This has already proved its value in service on such duties as the sterilizing of drug culture vessels and pipelines in the pharmaceutical field.

In the plant shown, demineralized water is pre-heated in the smaller HXU Paraflow (foreground) and is then pumped via the separator, which acts as a buffer tank, to the R.56 Paraflow. Here the feed is partly converted to steam by ordinary boiler steam at 3.2kg/cm² (45 lb/in²). The mixture of steam and boiling water passes to the separator where the water falls to the bottom and the steam passes out at the top. The condensate from the boiler steam is used as the heating medium in the pre-heater.

The plant illustrated was designed to generate 540kg/h (1,200 lb/h) of pure steam from demineralized water at a pressure of 1.4kg/cm² (20 lb/in²).

Components of Whisky, Wine and Beer

In the *Journal of the Association of Official Analytical Chemists*, **52**, No. 6, J. H. Kahn of the Research Department, National Distillers and Chemical Corp., 1275 Section Road, Cincinnati, Ohio 45237, has listed components which have been identified in alcoholic beverages by his laboratory and by other workers. The pertinent references are given.

Composition of Florida Orange Juice

In the *Journal of the Association of Official Analytical Chemists*, **52**, No. 6, pp. 1150/2, a statistical study and authentic data are given on authentic samples of Florida orange juice. These include soluble solids, acidity, amino acids, polyphenolics, 1-malic acid, and betaine.

Hazelnut Flavour

Naarden News, December 1969, **20**, No. 210, contains an article on 'Hazelnut Flavour'. There are also the regulations governing ice-cream in the Federal German Republic.