

# A New Method of Colorimetric Determination of Piperine Using *p*-Nitrophenyl Diazonium Fluoborate in Pepper (*Piper Nigrum L.*)

by

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(Received January 21, 1974)

## *p*-Nitrophenyl Diazonium Fluoborate를 이용한 후추 Piperine의 새로운 비색정량법

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### Abstract

A survey of the available methods for the determination of piperine was made, and a new method, developed based upon alkaline hydrolysis at about 140°C followed by colorimetric determination of the liberated piperidine. Piperidine on treatment with *p*-nitrophenyl diazonium fluoborate reagent gives a red colored complex which has an adsorption maximum at 530nm. The method measures the total pungency in pepper and applicable to piperine of black pepper and oleoresin of black pepper. The advantage of the present method is that the pungent compounds can be determined in micro samples.

### Introduction

Pepper (*Piper nigrum L.*) is one of the most important spices used for both flavor and aroma. Chemical composition of pepper is volatile oil (1 to 3 percent) and non-volatile portion which includes the pungent constituents. The oil may be obtained by steam distillation of dried black pepper and this represents the odorous constituents of the spice.

Oleoresins are solvent extracts of spice powders and contain non-volatile resinous material and the pungent constituents, in addition to the volatile essential oil.

The solvents commonly employed are: ethylene dichloride, ethanol, acetone, methylene dichloride, ethanol, acetone, methylene chloride, etc. The oleoresin is a popular flavoring material, since it closely resembles the original spice. The oleoresin of pepper would contain the volatile oil, some residual solvent, the pungent constituents, trace quantities of sugars, resinous material, fixed oils, polyphenols, pigments, etc.

The quantitative composition of the oleoresin depends on the solvent used and the variety of pepper, etc.

Pungent constituents in pepper are piperine, chavicine<sup>(1)</sup>, piperettine<sup>(2)</sup>, piperanine<sup>(3)</sup>, etc. The quality of pepper (and also the oleoresin) is, therefore, dependent on the amount of the pungent constituents present in it.

Taste characteristics of the pungent constituents were investigated as follows. Staudinger et al<sup>(4)</sup>, pointed out that the amide linkage with the aromatic acid is the factor responsible for pungency.

Recently, Kulka<sup>(6)</sup> reviewed the important aspects contributing to pungency and stated that piperine is predominantly responsible for bite in black pepper. He proved that the two double bonds and the methylene dioxy group present in the piperine do not contribute in any way to pungency.

A comparative picture of the bite intensities of the different pungent constituents is not available in literature. However, piperanine is reported to possess the bite intensity which is one half of piperine<sup>(5)</sup>.

There is a report that synthetic chemicals like 1-cinnamoyl piperidide<sup>(6)</sup> are used as adulterants in the oleoresin of pepper in U.S.A. This synthetic chemical possesses pungency similar to piperine.

The quality of pepper and also of the oleoresin of pepper is dependent on the amount of piperine (plus other pungent principles) present in it. The value of pepper is, therefore, dependent on the total content of the pungent constituents.

Hence, determination methods of the pungent constituents<sup>(7)</sup> are becoming more important. Previous workers<sup>(7-22)</sup> have reviewed the available methods and pointed out their merits and demerits. Therefore, the author reviewed publications about the pungent principles in pepper and developed the new method for determining piperine in pepper.

## Experiment

### 1. Materials

- (1) Black pepper: A sample available in the local market in India was finely ground, and used.
- (2) Oleoresin of black pepper: This sample was prepared in connection with other studies<sup>(23)</sup>.
- (3) Piperine: This was a gift sample obtained from the Griffith Laboratories, Canada. It melted at 129~130°C, and showed an absorption maximum at 345 nm.
- (4) 2N-Potassium hydroxide in propylene glycol: 112 g of potassium hydroxide (AR. quality) was dissolved

in 80 g of water and diluted to 1 liter with propylene glycol (BDH, b.p. 186~188°C).

- (5) *p*-Nitrophenyl diazonium fluoborate solution (PNP DF): This was prepared by dissolving 0.04 g of solid yellow substance in 100 ml acetone. It is necessary that the reagent should be prepared freshly before use. (Eastman Organic Chemicals Distillation Products Industries, Rochlster 3, New York, Div. of Eastman Kodak Co.)
- (6) Mixed Indicator: This was prepared by dissolving 0.1 g of methyl red and 0.5 g of bromocresol green in 100 ml ethanol.
- (7) Spectrophotometer: Absorption measurements were made with a Spectronic-20 colorimeter (Bausch and Lomb).

### 2. Methods

#### (1) Preparation of a calibrated chart

A known weight of piperine (about 0.05 g) was transferred into a 250 ml round bottomed flask. About 30 ml of 2N KOH in propylene glycol were added to this and refluxed using a Liebig's condenser for 2 hours on a sand bath maintaining the temperature around 140°C. The mixture was cooled to the room temperature. About 100 ml of water and a few drops of antifoam reagent (emulsified silicone compound) were added to the flask. The reflux condenser was removed and the flask was connected to a distillation set. The mixture was heated to distill off the piperidine which was absorbed in acetone(5 ml).

The distillation was stopped when the distillate was neutral to indicator paper (pH 7.0). The distillate was divided into two equal portions-(a). The first portion (about 40 ml) was titrated with 0.01N HCl using the mixed indicator, according to Labruyere<sup>(5)</sup>. The amount of piperidine was calculated and the corresponding weight of piperine computed, using the following equation.

$$\text{Piperine(\%)} = \frac{\text{wt. of piperidine} \times 3.353 \times 100}{\text{wt. of piperine in gram.}}$$

$$*3.353 = \frac{\text{Molecular weight of piperine (285.35)}}{\text{Molecular weight of piperidine (85.15)}}$$

- (b) The second portion (about 40 ml) was diluted with acetone (to a volume of 50 ml). From this, measured volumes in the range 2 to 6 ml, were transferred to different test tubes. To each of these tubes was added 2 ml of *p*-nitrophenyl diazonium fluoborate. A red color was obtained and these were suitably diluted (10 ml) using acetone. The absorbance values

were measured in the region 480 to 600 nm and the absorption maximum was found at 530 nm (Fig. 1).

The absorbance readings of the color complex were measured ( $\lambda_{max}$  530 nm) after every 10 minutes for a period of 2 hours and the results showed no change in the color.

The values of absorbance of the different solutions were measured at 530nm using a reagent blank (2 ml water+2 ml reagent diluted to 10 ml with acetone). The data (absorbance values vs piperine ( $\mu$ g)) are represented in figure 2. A weighed quantity of piperine was analysed by the above colorimetric method and the purity determined (Table 2).

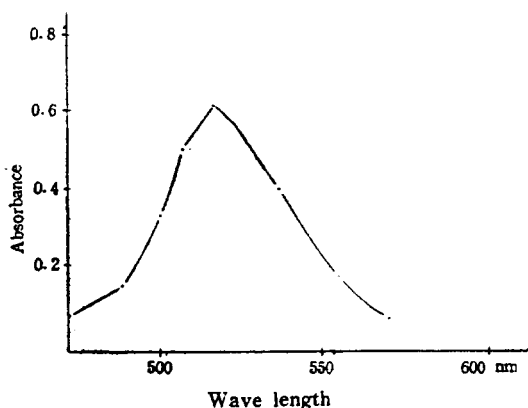


Fig. 1. Spectral pattern of the colored complex

(2) Determination of pungent constituents in black pepper and oleoresin of pepper

- (a) Black pepper: 2 grams of ground black pepper were extracted with about 100 ml acetone in soxhlet apparatus for 20 hours. The solvent acetone was removed by vacuum distillation to obtain a green syrupy liquid of oleoresin of pepper. This was hydrolysed with propylene glycol containing KOH and the piperidine determined (expressed as piperine) by methods described above (i.e. the titrimetric and the *p*-NPDF methods).
- (b) Oleoresin of black pepper: An accurately weighed sample of about 0.5 g of well homogenized oleoresin of pepper was transferred to a round bottomed flask. It was refluxed for 2 hours using the propylene glycol containing 2 N KOH and the piperidine containing pungent constituents determined by both the titrimetric and colorimetric methods.

**Results and Discussion**

The survey of available methods for the determina-

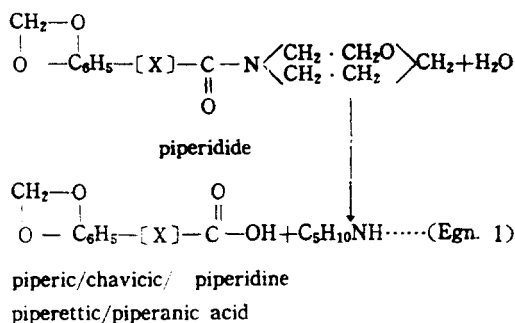
tion of the pungent constituents clearly shows that no one method is free from defects. Recently, the ISO examined critically the usefulness of ultraviolet spectroscopy<sup>(16)</sup> and volumetric methods<sup>(8)</sup> and recommended the U.V. method as acceptable. In order to obtain a realistic and useful result, one or more methods should be employed which give complimentary data. A comparative data of the piperine content determined by various methods are given in Table 1.

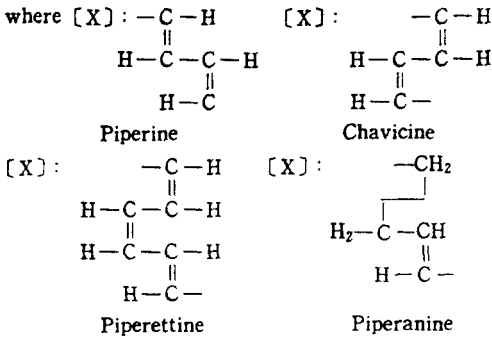
Table 1. Piperine content in pepper samples

Method used	Amount of piperine (%)	
	Black pepper	Oleoresin
1. U.V. spectrometry <sup>(16)</sup>	6.7	52.6
2. Chromotropic acid <sup>(19)</sup>	7.8	—
3. HNO <sub>3</sub> -KOH-Thiourea <sup>(20)</sup>	7.6	—
4. Polarographic <sup>(14)</sup>	—	50.1
5. Kjeldahl <sup>(10)</sup>	—	67.3

No study seems to have been made correlating the objective and subjective evaluation of pungency in pepper samples.

The colorimetric method<sup>(21)</sup> developed in CFTRI laboratory measures the total pungencies (piperine+piperettine+chavicine+piperanine) whereas, the U.V method determines either piperine or piperine+piperettine. In the above colorimetric method, the piperidides are hydrolysed to liberate piperidine which is reacted at 0°C, with carbon disulphide to form piperidinium pentamethylene dithiocarbamate (PPDC). The PPDC reacts with cupric sulfate to give a colored complex ( $\lambda_{max}$  435 nm) which obeys Beer's law between a concentration of 1 to 14  $\mu$ g/ml of piperidine. The proposed method also employs alkaline hydrolysis of the piperidides (Equation 1) to yield piperidine which is subsequently reacted with *p*-nitrophenyl diazonium fluoroborate (PNPDF) to give a red colour ( $\lambda_{max}$  530 nm).





The curve of figure 1 shows the spectral patterns of the colored complex reacting with piperine and p-nitrophenyl diazonium fluoroborate solution in acetone. Absorption maxima was observed at 530 nm. Various concentrations of pure piperine in *p*-NPDF-acetone solution were examined from 400~600 nm. The largest maximum was noted at 530 nm. Absorbance values at 530 nm. Absorbance values at 530 nm for concentrations from 1500 to 4000  $\mu\text{g}/100 \text{ ml}$  were plotted in Fig. 2.

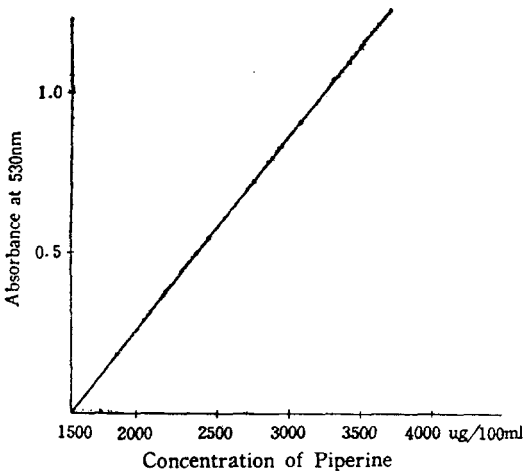


Fig. 2. Absorbance (530 nm) concentration of piperine

The recovery of pure piperine in *p*-NPDF-acetone solutions of piperine using the new colorimetric method (*p*-NPDF) is recorded in Table 2.

Table 2. Analysis of piperine

Weight piperine taken in gm	Hours of reflux	Recovering rate of piperine (%)	
		By titrimetric method (8)	By the <i>p</i> -NPDF method
1) 0.047	2.0	99.72	98.87
2) 0.046	2.0	99.81	98.79
3) 0.048	2.0	99.85	98.81

The recovery rate ranged from 98.79 to 98.87 percent. Table 2 showed a fairly good agreement between the titrimetric and colorimetric methods. A comparison of the present method with the titrimetric method is shown in Table 3.

Table 3. Analysis of black pepper and oleoresin of black pepper

Sample	Weight taken in gm	Pungent constituents found in percent (expressed as piperine)	
		Titrimetric method	<i>p</i> -nitrophenyl diazonium fluoroborate method
Black pepper	1) 2.0	5.78	5.61
	2) 2.0	5.79	5.53
	3) 2.0	5.51	5.27
Oleoresin of black pepper	1) 0.5	55.96	55.74
	2) 0.5	55.46	55.07
	3) 0.5	55.32	55.22
	4) 0.5	55.40	55.19
	5) 0.5	55.27	55.01
	6) 0.5	55.39	55.27

\*Distillate collected: 140 ml

The present method gave results averaging 0.22 percent lower than the titrimetric method. The much greater difference, + 0.39 percent of the titrimetric method as compared to present method appears to be less significant. The higher values by the titrimetric method can be undoubtedly present in oleoresins of black pepper, such as chavicine, an uncrystallizable isomer of piperine <sup>(1), (2), (3)</sup>. these compounds are present only in small amounts in pepper<sup>(24)</sup>. The U.V spectrophotometric method is specific for piperine and not for other pungent like piperettine, chavicine, and piperanine which are also present in black pepper. therefore inclusion in the piperine determination as a measure of pungency leads to only a minor inaccuracy, because they also contribute to the pungency of the spice.

In the present method, the pungent constituents on hydrolysis yield (piperidine) reacts with *p*-nitrophenyl diazonium fluorate to give a stable red color. The advantage of the present method is that pungent principles can be determined in microsample of black pepper. The present method appears to be specific for total pungent constituents.

요 약

후추의 매운맛을 주는 자극성분인 piperine 정량에 대

한 가능한 방법들에 관하여 조사정리 하였고, 또 새로운 정량법으로 후추의 매운 성분을 140°C에서 알칼리 가수분해로서 유리된 piperidine을 *p*-nitrophenyl diazonium fluoborate reagent 와 반응처리 시킴으로써 530 nm에서 최고 흡광도를 보이며 안정된 적색 복합화합물을 생성시켜 이 복합물의 색을 colorimeter로 측정하는 비색정량법을 개발 하였다.

이 새로운 방법은 후추의 전 매운맛의 주성분인 piperine의 정량에 특히 유용하였다. 이 분석법의 장점은 일반 적정법보다 적은 양의 시료로서 후추에 함유된 자극 성분인 piperine의 측정이 가능한 것이다.



The author wishes to express his thanks to Dr. M. L. Shankaranarayana, Scientist, Discipline of Plantation Products and Flavor Technology, Central Food Technological Research Institute, Mysore, India for his constant encouragement throughout this work.

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