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Note

Determination of piperine in pepper *(Piper nigrum)* using high-performance liquid chromatography

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Pepper (*Piper nigrum* L), a major spice in world trade, is valued for its pungent taste and aroma. The pungent principles of pepper have been the subject of many investigations since the early 19th century. The knowledge of the chemistry of pepper dates back to 1820, when the most abundant alkaloid piperine was isolated[1]. Piperine or 1-piperoylpiperidine ($C_{17}H_{19}NO_3$), the pungent principle, together with other pungent substances present in small quantities such as chavicine, piperidine and piperettine are responsible for the sharp biting taste and pungency. In addition, pigments, resins, sugars and fixed oils may also be found in the non-volatile ether extract. Since piperine is universally accepted as the predominant pungent principle in pepper, the quality of pepper and also of the oleoresin is dependent largely on the piperine content and thus methods for estimating piperine are becoming more important[1–3].

Methods available for analysis of piperine include Kjeldahl nitrogen determination[4], adaptation of the chromotrophic acid test for formaldehyde[5], colorimetric methods using nitric acid[6], sulphuric acid and aromatic aldehyde[7], phosphoric acid[8] and based on alkaline hydrolysis[9], reaction with *p*-nitrophenyl diazonium fluoborate[10], volumetric analysis[11], spectrophotometric analysis[12,13] and high-performance liquid chromatography (HPLC)[14–17].

Graham[7] and Labruyere[9] have reviewed the available methods and pointed out their merits and demerits. The Kjeldahl and UV-spectrophotometric methods have been widely and frequently practised. The Kjeldahl method, however, measures other nitrogenous compounds and always gives high values[12,13,16,18,19]. The UV-spectrophotometric method has been developed for the direct measurement of piperine and is very specific and reliable under controlled conditions[12,13,16,18,19]. Recently HPLC was selected as a rapid, sensitive and specific method for the quantitative determination of piperine, because of its ease of operation and proven ability to detect and separate the small quantities of non-volatile, UV-active components[14–17].

In this study we have used a reversed-phase μBondapak CN column and a mobile phase of methanol–water to develop a method for the rapid analysis of piperine in pepper and non-volatile ether extracts. The piperine levels were compared with those obtained using a UV method.

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EXPERIMENTAL

Samples and chemicals

An authentic sample of piperine was purchased from Sigma (St. Louis, U.S.A.).

Black, green and white pepper samples of the Kuching variety, harvested at different maturities were obtained from the Minor Export Crops Research Station, Matale, Sri-Lanka.

All chemicals were of analytical-reagent grade. Water was double distilled in glass.

HPLC conditions

The equipment used was manufactured by Waters Assoc. (Milford, MA, U.S.A.) and consisted of a Model M 6000A solvent delivery pump and Model 440 absorbance UV detector set at 280 nm, a stainless-steel μBondapak CN column (30 cm × 3.9 mm I.D.). The mobile phase was methanol (Waters Assoc., chromatographic grade) and water which had been filtered through a Sartorius filter (0.45 μm pore size, SM 11106) and degassed under vacuum. A flow-rate of 2 ml/min, attenuation of 0.5 a.u.f.s. and a chart speed of 1 cm/min were used. Samples (10 μl) were injected directly after filtration.

Some experiments were carried out using a Waters Model 480 variable-wavelength UV detector set at 345 nm using the same separation conditions as above except that attenuation was 2.0 a.u.f.s. and sample size 5 μl.

Extraction

Pepper samples were ground to a fine powder in a coffee grinder and extracted (5.00 g) with diethyl ether in a Soxhlet apparatus for 20 h. The solvent ether was removed by vacuum distillation and the extract heated to constant weight at 100°C.

Spectrophotometric procedure

The method developed by Fagen *et al.*[12], was used. A well-mixed sample of non-volatile ether extract of pepper (0.25 g) was dissolved in 100 ml chloroform and an aliquot (0.5 ml) further diluted to 100 ml. The flasks were covered with aluminium foil to protect the samples from photoisomerisation. The absorbance at 345 nm was measured immediately using chloroform as a blank and the proportion of piperine in the non-volatile ether extract calculated with reference to a calibration curve over the range from 0.2 mg to 1.2 mg piperine in 100 ml chloroform.

HPLC procedure

A well-mixed sample of non-volatile ether extract of pepper (10 mg) was dissolved in 20 ml methanol and filtered through a Sartorius membrane filter (0.45 μm pore size, SM 11806) before injection. The four mobile phases which were tested with the pure piperine and pepper samples were (i) double distilled water, (ii) methanol–water (50:50), (iii) methanol–water (80:20), and (iv) methanol. Methanol–water (50:50) was used in subsequent experiments.

Pure piperine (10–50 mg) was dissolved in 100 ml methanol and aliquots (10 μl) injected into the HPLC. Calibration graphs were plotted with peak height against

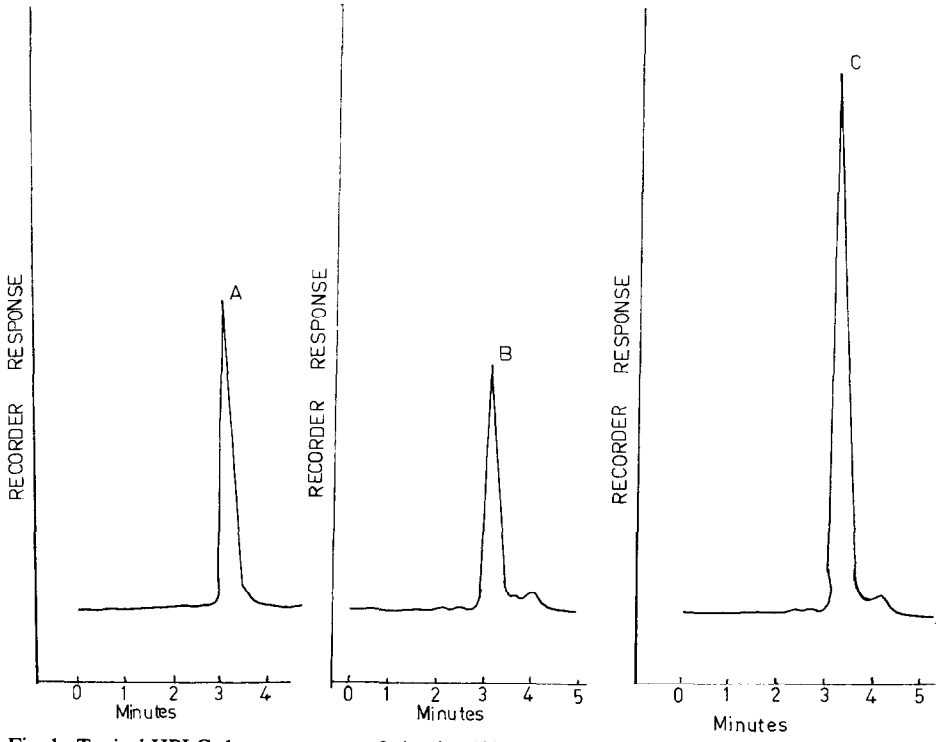

Fig. 1. Typical HPLC chromatograms of piperine (A); pepper extract (B) and pepper extract spiked with piperine (C), detected at 280 nm.

TABLE I

PIPERINE LEVELS IN NON-VOLATILE ETHER EXTRACT OF PEPPER

Values in %.

Sample	*Age of berries (months)*	*Spectrophotometric method*	*HPLC method**
Black pepper	2	35.4	32.4
	3	47.2	44.1
	4	50.5	49.7
	5	49.5	47.6
	6	47.9	44.1
Green pepper	4	53.6	53.1
	4½	55.2	54.2
	5	51.6	49.7
	5½	50.6	48.3
White pepper	5	47.2	44.1
	5½	45.7	43.5
	6	42.6	41.4

* Detection at 280 nm.

TABLE II

RECOVERY OF PIPERINE ADDED TO NON-VOLATILE ETHER EXTRACT BY SPECTROPHOTOMETRIC AND HPLC METHODS

Samples	*Spectrophotometric method*			*HPLC method at 280 nm*			*HPLC method at 345 nm*		
	Added (mg)	*Found (mg)*	*Recovery (%)*	*Added (mg)*	*Found (mg)*	*Recovery (%)*	*Added (mg)*	*Found (mg)*	*Recovery (%)*
Black pepper	5.00	4.60	92.0	4.75	4.75	100.0	6.30	6.26	99.4
	10.00	9.60	96.0	7.85	7.79	99.2	9.15	9.03	98.7
Green pepper	5.00	4.80	96.0	4.75	4.69	98.7	4.35	4.33	99.5
	10.00	9.80	98.0	7.85	7.87	100.4	8.50	8.47	99.7
White pepper	5.00	4.80	96.0	4.75	4.82	101.6	5.15	5.14	99.8
	10.00	9.40	94.0	7.85	7.78	99.1	8.97	8.95	99.8

piperine concentration detected at both 280 and 345 nm. Linear relationships were found over the range 10 to 40 mg piperine/100 ml methanol.

RESULTS AND DISCUSSION

Fig. 1 shows the chromatograms of pure piperine, a pepper sample and a pepper sample spiked with piperine and using the selected mobile phase and piperine detection at 280 nm. At a flow-rate of 2.0 ml/min piperine was eluted after 3 min; considerably faster than HPLC separations reported previously[15–17] and also using a simple and cheap mobile phase.

Results of spectrophotometric and HPLC (280 nm) methods of analysis of piperine are given in Table I. Although the difference in piperine levels determined by both methods is small, the spectrophotometric method always gave slightly higher results than did the HPLC method. Interference by other nitrogenous components such as piperettine may be the reason for the higher values with the UV method.

The recovery of piperine by both methods shown in Table II demonstrates that the present HPLC procedure gives a higher and less variable recovery for all samples tested, as well as rapid and inexpensive separation. Of considerable interest is the increased sensitivity of the HPLC method when detection at 345 nm is used compared to detection at 280 nm. The method was found to be nearly 8 times as sensitive when HPLC separation was coupled with detection at 345 nm. Thus availability of a variable-wavelength detector further improves the value of the HPLC method described when compared with the traditional UV-spectrophotometric methods.

Further work will establish the variability of piperine levels in several pepper varieties at different stages of maturation, processing and storage.

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