METHODS FOR THE INSTRUMENTAL ANALYSIS OF THE COMPONENTS OF A CO_2 EXTRACT OF Piper nigrum

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One of the most important spice crops is Piper nigrum L. (black pepper), which is widely used in food products both abroad and in the USSR [1, 2]. The production of extracts from spice and aromatic plants, including black pepper, by their extraction with liquefied carbon dioxide has been organized in the experimental factory of the Krasnodar Scientific-Research Institute of the Food Industry [3, 4].

The main component responsible for the sharp burning taste of pepper is the alkaloid piperine, which possesses the property of absorbing in the near-UV region at 240-400 nm [5, 6]. It has been isolated from many species of pepper in the form of crystals with mp 129-130°C.

In addition to piperine, pepper contains its isomer chavicine [7] and also piperettine, piperonal and other substances [8, 9] which likewise cause a burning taste, but to a smaller degree.

Several methods for determining the amount of piperine in the fruit of black pepper are known:

1) An indirect method based on the Kjeldahl determination of nitrogen [8];

2) a colorimetric method using chromotropic acid [10];

3) a colorimetric method using sulfuric acid and p-dimethylbenzaldehyde [11];

4) a colorimetric method using phosphoric acid [12]; and

5) a hydrolytic method [13].

The standard method of estimating the amount of piperine in the fruit of black pepper is based on the Kjeldahl determination of its nitrogen content, which is calculated to piperine. However, this method gives high values because of the presence of other nitrogen-containing substances. Unsaturated fats and some components of the essential oils also have an unfavorable influence on the results of methods 2) and 3). In the case of method 4) based on phosphoric acid, inadequately stable colored derivatives are formed, which complicates its use. The hydrolytic method, based on alkaline hydrolysis at 138°C with the subsequent titration of the piperine liberated, is very laborious and time-consuming. To study some processes of extraction with liquid carbon dioxide it is necessary to use micro methods. We have developed spectrophotometric and colorimetric methods of determining the amounts of piperine and of the combined bitter substances.

EXPERIMENTAL

The solvents and reagents (ethanol, isopropanol, ethyl acetate, diethyl ether, hexane, heptane, thiourea) used for the development of the methods were subjected to additional purification before use (redistillation, fractional recrystallization). The UV-spectrometric determinations were performed on an SF-4a spectrophotometer. The colorimetric method was developed with the use of an FÉKM-56 photoelectric colorimeter with a blue filter.

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To identify the alkaloids and the combined bitter substances of the CO_2 extract of pepper we used ascending thin-layer chromatography in a nonfixed layer of alumina (Brockmann activity grade II) with a layer thickness of 0.5 mm in the chloroform–ethanol (1 : 10) solvent system with iodine vapor or the Dragendorff reagent to reveal the spots [14]. The relative amounts of the individual components in the mixtures were judged from the areas of the spots on the chromatograms, and they were identified by comparison with markers.

It was found that in the CO_2 extract of pepper the piperine is present partially in the form of crystals and partially in the form of a solution in the liquid extract. Part of the piperine is present in the resinous part of the extract in the form of its di-cis stereoisomer chavicine. Pure piperine for use as a standard was prepared in the following way: the CO_2 extract of black pepper was left for a day at +3 to +5°C, and then hexane or heptane (1 : 7) was added to it and the mixture was carefully stirred. The crystalline precipitate that separated out was filtered off through a Schott 3 or 4 filter. The residue on the filter was washed with hexane or heptane and was subjected to repeated recrystallization first from ethanol and then from isopropanol and was passed through a layer of alumina (activity grade II).

The crystals of piperine obtained were pale yellow with mp 128.5-129°C and gave the following color reactions: with phosphoric acid in the presence of conc. sulfuric acid and with p-dimethylbenzaldehyde and a positive Labat reaction – a blue color for methylenedioxy groups [11, 12, 15]. The spectrum of piperine (ethanol) in the UV region was identical with the spectrum of the CO_2 extract of pepper and its liquid fraction (Fig. 1). As can be seen from Fig. 1, absorption maxima appear at wavelengths of 260, 310, and 342 nm.

A sample of the CO_2 extract of pepper weighing between 0.02 and 0.05 g was dissolved in absolute ethanol. The optical density was measured on the SF-4A instrument at a wavelength of 343 nm. The amount of piperine in the CO_2 extract (%) was calculated from the formula

$$Y = \frac{0.8390 \cdot D \cdot n \cdot 100}{P},$$

where D is the optical density of the solution of the extract in ethanol; n is the dilution used in the experiment; P is the weight of the CO_2 extract, g; and 0.8390 is a calculating factor the numerical value of which is equal to the tangent of the angle of slope of the calibration curve to the axis of abscissas.

The calibration curve was plotted for pure piperine.

The results of the determination of the amount of piperine in a CO_2 extract of black pepper were compared with the results of a Kjeldahl determination.

The results of a determination of the amount of piperine and of the total amount of bitter substances in a CO_2 extract of pepper (% of the sample) by several methods are given below:

	Spectrophotometric	Colorimetric	Kjeldahl
	40.25	55.9	59.60
	40.46	55.2	59.90
	40.27	55.6	60.20
	40.02	55.8	60.10
	40.70	55.78	60.00
Mean	40.34	55.66	59.96

The investigations showed that the relative error of the spectrophotometric method does not exceed 2%, while that of the Kjeldahl method is 7%.

For an objective evaluation of the total amount of bitter substances in the CO_2 extract of pepper we have developed a colorimetric method based on the property of the bitter substances of black pepper of forming with concentrated nitric acid yellow quinoid complexes which give a dark red color on the addition of alkali [16].

A sample of the CO_2 extract of pepper of between 0.10 and 0.04 g was dissolved in 10 ml of freshly distilled diethyl ether or ethyl acetate. The solution of the extract (1 ml) was transferred to test tubes or measuring flasks which were placed in the water bath for the evaporation of the ether. After cooling, to each test tube was added 0.6 ml of concentrated nitric acid and the mixture was kept in the water bath at 40°C for 10 min and was then poured into 5 ml of a 40% aqueous solution of caustic potash, and the resulting mixture was placed in the boiling water bath for 5 min. The contents of the test tubes were cooled to room temperature and to each was added 10 ml of a 6% aqueous solution of thiourea, after which an intense red

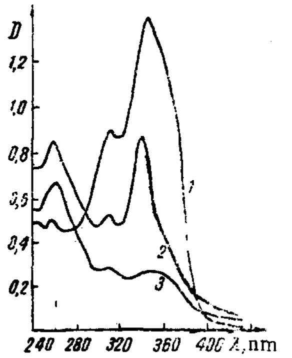

Fig. 1

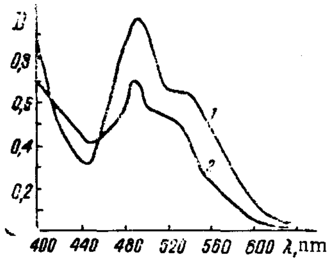

Fig. 2

Fig. 1. Characteristics of the UV spectra of piperine (1), a CO_2 extract of pepper (2), and the liberated fraction of the extract (3).

Fig. 2. Spectral curves of the reaction of the products with concentrated nitric acid and 40% alkali: 1) piperine; 2) CO_2 extract of pepper.

coloration appeared. The optical densities of the colored solutions of different concentrations of combined bitter substances and of the CO_2 extract were measured in comparison with a blank reagent prepared simultaneously without the addition of the product under investigation. It can be seen from a consideration of the spectra of the solutions mentioned (Fig. 2) that the maximum on the absorption curves is at a wavelength of 490 nm. The calibration curve was constructed for the combined bitter substances isolated by the method described above.

The amount of combined bitter substances in the CO_2 extract was determined from the formula (%)

$$X = \frac{K \cdot D \cdot v \cdot 100}{P},$$

where D is the optical density of the products of the reaction of the extract with concentrated nitric acid and 40% alkali; v is the volume of the dilution used in the experiment; P is the weight of the CO_2 extract, g; and K is a calculation factor determined from the calibration curve plotted for the combined bitter substances and representing the numerical value of the tangent of the angle of slope of the calibration curve to the axis of abscissas. The methods that have been developed for determining the amounts of piperine and of total bitter substances in the CO_2 extract have also been used to determine these substances in the dry fruit of bitter black pepper.

A sample of 0.1-0.5 g of peppercorns was ground to a powdery state and was steeped for 2 h in 25 ml of ethanol or ether. Then the extract was filtered and the subsequent determination was performed by the methods described above.

SUMMARY

1. A method for isolating the pure alkaloid piperine from a CO_2 extract of black pepper has been developed

2. Methods of instrumental (spectrophotometric and colorimetric) analysis have been developed which permit the determination of the amounts of piperine and of total bitter substances in a CO_2 extract of pepper and in peppercorns.

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