dryness to saturation appears to fall into three stages, thus giving rise to the sigmoid-type curve when relative humidity is plotted against moisture content. For rough rice the first stage extends from dryness to approximately 7% moisture content (30% relative humidity) and this point is characterized by an inflection point. Water is adsorbed as a unilayer throughout this range and has been considered as such by other investigators (2–4, 10, 11). The second stage of adsorption extends from approximately 7 to 14% moisture content (30 to 65% relative humidity) and represents the formation of a second molecular layer of water. This intermediate portion of the curve is linear, indicating a uniform increase of moisture content with increasing relative humidity. Beyond the intermediate adsorption stage, a condensed water film is formed above the second layer. As the moisture content increases through the range above 14%, the condensed film grades into a liquid water layer at 100% relative humidity and the heat of adsorption of water by rough rice decreases, approaching that of a free water surface.

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SPICE ANALYSIS

Spectrophotometric Method for Determining Piperine in Oleoresins of Black Pepper

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THE ALKALOID PIPERINE is generally accepted as the active "bite" component in black pepper (3, 4). The present chemical method for estimating the piperine content of oleoresins of black pepper is based on the determination of total nitrogen by the Kjeldahl-Wilfarth-Gunning method (7). However, this method measures "crude" piperine and is subject to variations due to the presence of other nitrogenous substances.

As piperine absorbs in the ultraviolet region with a maximum at 345 mμ (9), a simple, rapid, quantitative method is proposed involving absorbance measurements at this wave length.

Isolation of Pure Piperine

The piperine used as a standard in the proposed spectrophotometric method is isolated and purified by the following method.

To 100 grams of an oleoresin of black pepper add 500 ml. of petroleum ether, mix well, and filter. Dissolve the pre-

Table I. Recovery of Piperine by Spectrophotometric Method

No.	In Oleoresin, G./100 Ml.	Added, G./100 Ml.	Found, G./100 Ml.	Recovered, %
1	0.00062	0.00010	0.00072	100.0
		0.00020	0.00081	98.7
		0.00030	0.00089	96.7
		0.00040	0.00100	98.0
2	0.00039	0.000125	0.00053	102.9
		0.000250	0.00065	101.5
		0.000375	0.00078	101.8
		0.000500	0.00089	100.0

cipitate in a minimum quantity of hot absolute ethyl alcohol, filter, and allow the crude piperine to crystallize out as long needles. Recrystallize the piperine three times from hot absolute ethyl alcohol. The melting point of the recrystallized piperine was 130° C. (Fisher-Johns melting point apparatus). The reported melting point for pure piperine is 129–130° C. (8). Elemental analysis

showed the isolated piperine to be carbon 71.90% and hydrogen 6.64%. Theoretical: carbon 71.55% and hydrogen 6.71%. With concentrated sul-

Figure 1. Spectral absorbance of pure piperine and an oleoresin of black pepper

1. Piperine, 0.0005 gram in 100 ml. of chloroform
2. Oleoresin, 0.00125 gram in 100 ml. of chloroform
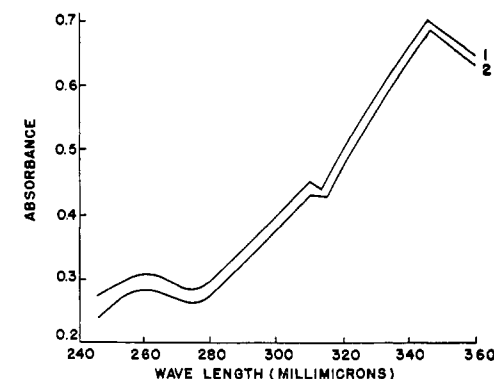
The chemical method for estimating piperine—believed to be the active "bite" component in oleoresins of black pepper—is based on the determination of total nitrogen by the Kjeldahl-Wilfarth-Gunning method. Inasmuch as it measures "crude" piperine, subject to variations due to the presence of other nitrogen compounds such as chavicine, a simple, rapid, and specific quantitative method was needed to estimate the pungency of oleoresins based on piperine content. The ultraviolet absorption spectrum of an oleoresin of black pepper in chloroform is identical to that of pure piperine in chloroform. Maximum absorption was observed at 260, 310, and 345 mμ. The spectrophotometric method adopted involves measurement of absorbance at 345 mμ. Absorbance measurements at 345 mμ when plotted against concentration showed excellent conformity to Beer's law. The recovery of pure piperine in oleoresins was from 96.7 to 102.9%.

furic acid the isolated piperine gave a blood-red color (6). It also gave a positive reaction in the Labat test for the methylenedioxy group.

Spectrophotometric Method

Apparatus and Reagents. Redistilled chloroform, c.p. Beckman spectrophotometer, Model DU, with ultraviolet attachment. 1-cm. silica cells.

Table II. Comparison of Spectrophotometric, Polarographic, and Kjeldahl-Wilfarth-Gunning Methods

	Piperine %		
Sample	Spectrophotometer	Polarograph	Kjeldahl-Wilfarth-Gunning
Ceylon	52.6	50.1	67.3
	47.7	43.4	60.1
Malabar	45.0	42.4	57.9
	46.7	45.6	53.3
	43.2	41.4	56.5
	45.7	44.5	57.4
	44.6	38.9	53.6
	41.8	41.3	51.8
	42.1	38.8	52.9
	40.2	40.8	52.6

All values are averages of duplicate determinations.

Procedure. Accurately weigh a 0.25-gram sample of a thoroughly mixed oleoresin of black pepper, transfer to a 100-ml. volumetric flask with redistilled chloroform, and make up to volume. Pipet a 0.5-ml. aliquot into another 100-ml. volumetric flask and make up to the mark with chloroform. Immediately transfer a portion of the final solution to a 1-cm. silica cell, using a matched cell containing redistilled chloroform as the reference. Observe the absorbance value at 345 mμ, using a Beckman spectrophotometer, Model DU, with an ultraviolet attachment. Refer to a calibration curve for piperine in chloroform to obtain the per cent concentration in the final solution, and make the necessary correction for the aliquot taken to obtain

the percentage of piperine in the original sample.

Results and Discussion

The curves in Figure 1 show that the ultraviolet absorption spectrum for an oleoresin of black pepper in chloroform is identical to that of pure piperine in chloroform. Absorption maxima were observed at 260, 310, and 345 mμ.

Various concentrations of pure piperine in chloroform were examined from 240 to 360 mμ. The largest maximum was noted at 345 mμ. Absorbance values at 345 mμ for concentrations from 0.00025 to 0.00125% were plotted, and a working curve, showing excellent conformity to Beer's law, is shown in Figure 2. The recovery of pure piperine in chloroform solutions of oleoresins using the spectrophotometric method is recorded in Table I. Recoveries ranged from 96.7 to 102.9%.

A comparison of the spectrophotometric with the Kjeldahl-Wilfarth-Gunning method and the polarographic method of Hanc and Santavy is shown in Table II (4, 5). The spectrophotometric method gave results averaging
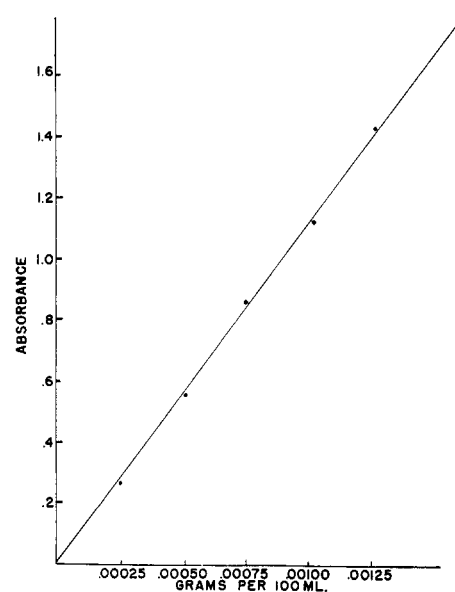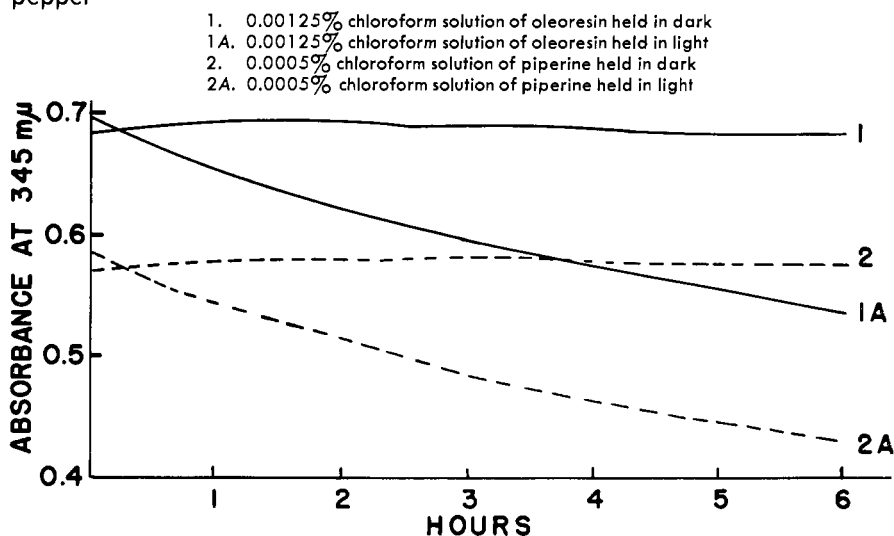
2.24% higher than the polarographic method. The much greater average difference, +11.39%, of the total nitrogen method as compared to the spectrophotometric method appears to be

Figure 2. Absorbance (345 mμ) vs. concentration of piperine

Figure 3. Effect of light on dilute solutions of piperine and oleoresins of black pepper

1. 0.00125% chloroform solution of oleoresin held in dark
1A. 0.00125% chloroform solution of oleoresin held in light
2. 0.0005% chloroform solution of piperine held in dark
2A. 0.0005% chloroform solution of piperine held in light

highly significant. The higher values by the Kjeldahl - Wilfarth - Gunning method can be undoubtedly attributed to other nitrogen-containing compounds reportedly present in oleoresins of black pepper, such as chavicine, an uncrystallizable isomer of piperine (2, 7).

Figure 3 shows the effect of light on dilute solutions of piperine and oleoresins of black pepper. It is apparent from these data that piperine is photosensitive in dilute solutions of chloroform; therefore, spectrophotometric measurements should be made immediately for accurate results.

Several advantages may be cited for the spectrophotometric method as applied to oleoresins of black pepper. It

is both simple and rapid, requiring no time-consuming digestion, as in the case of the total nitrogen method. The spectrophotometric method appears to be specific for piperine. In contrast, the estimation of piperine by total nitrogen would be subject to error due to the presence of other nitrogen compounds in oleoresins of black pepper.

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SUGAR DETERMINATION

Rapid Sugar Extraction Procedure for Analysis of Candied Fruits, Jams, and Fresh Fruits

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A rapid sugar extraction procedure based on use of a Waring Blendor was adapted to the analysis of candied fruits, jams, strawberries, and stone fruits. Total and reducing sugar contents as determined by this method were compared with results by a modified AOAC method for fruit and fruit products. Statistical analyses show that the methods are equivalent in precision and accuracy when applied to analysis of candied fruits, fresh strawberries, apricots, and prunes. In analysis of jams, the rapid method gives results 0.24% higher for total sugars but shows no significant difference for reducing sugars. A complete determination of total and reducing sugars may be made inexpensively in less than 1 hour by the rapid method, whereas the modified AOAC method requires 2.5 hours.

IN PREPARING JAMS AND CANDIED FRUITS using sucrose and invert sugar, it is essential that a careful control be maintained on the total sugar concentration as well as on the ratio of sucrose to invert sugar. The latter relationship is necessary to prevent crystallization. In the manufacture of candied fruits by the continuous evaporation process of Atkinson and others (4), and in jam production (13), analyses of sirup and fruit at regular intervals assist in maintaining uniformity and stability in the finished product. There is a definite need, particularly under factory conditions, for an accurate, rapid, inexpensive procedure for sugar determination to replace the present standard methods for both processed and fresh fruit. A simple plant control method developed by two of the writers (17) for candied fruit operations proved so successful that it was investigated further with a view to increasing its accuracy and adapting it to other products.

The standard procedure employed

by the authors for accurate determination of sugar in fruits and fruit products involves two minor modifications of the Association of Official Agricultural Chemists method for fruits and fruit products (1-3). Prior to extraction by boiling (1) sufficient 1N sodium hydroxide solution is added (with vigorous stirring) to neutralize the natural acid. This minimizes the inversion of sucrose and consequent high values for reducing sugars. The solution should have a pH of 6 to 7 at the end of the boiling extraction period. For inversion of sucrose in the determination of total sugars as invert, citric acid is substituted for hydrochloric acid (1 + 1). Five grams of citric acid crystals are added to a 100-ml. aliquot and the solution is boiled gently for 10 minutes, cooled, and neutralized (8). This procedure has been found to give results comparable to those of the AOAC method (16).

Elimination of Clarification. Clarification of plant extracts with excess neutral lead acetate followed by precipi-

tation of excess lead with sodium oxalate has been adopted by the Association of Official Agricultural Chemists (2) as an official method for fruit and fruit products. Data presented by Morris and Welton (12), McDonald (10), Williams and others (19), and Williams and Bevenue (18) indicated that in many cases aqueous or alcoholic extracts of plant materials require no clarification prior to sugar analysis. Several of these authors reported that lead acetate precipitation does not remove all nonsugar reducing substances.

Elimination of Neutralization. The necessity for neutralization prior to boiling water extraction of sugars from acid products was noted by McRoberts (11) and Roy (15). Bates and others (5), however, presented data to indicate that inversion at room temperature or lower is negligible in the time interval required for performing a sugar extraction.

Blender Extraction. Although elimination of clarification and neutraliza-