samples. Each result represents the average of eight actual determinations. Each result reported using Olcott's method was obtained from the difference between the average of three amino nitrogen determinations on the autoclaved and the average of three similar determinations on the unautoclaved samples.

Discussion

The addition of 2-butanol to the buffered solvent recommended by McFarren (2) improves the separations of glutamic acid from the other amino acids, and leads to the formation of round, compact spots. Following the procedure described in this paper, glutamic acid was separated in the presence of relatively high concentrations of sodium chloride. The same solvent system has been used successfully for the determination of aspartic acid, serine, glycine, threonine, and alanine, as well as glutamic acid.

A heating period of 15 minutes at 60° C. is recommended for the rapid development of the ninhydrin color. Higher temperatures give spots that tend to fade quickly and are unsatisfactory for quantitative work, unless the color intensities are measured immediately following the heating period. Spots which do not fade appreciably in a week may be obtained by simply allowing the chromatograms to hang in the dark for 24 hours, instead of heating them at elevated temperatures.

The divergence between duplicate determinations made on a given hydrolyzate may be as high as 20%. In order to check this point, eight separate analyses were made on each of six hydrolyzates (series 1) and the standard errors were computed. The coefficient of variation was approximately 2.0% for sample C (54% glutamic acid), but rose to 6.5% for sample D (5.7% glutamic acid). In a similar analysis on a second series of hydrolyzates, similar values were secured. It may be concluded that eight replicate determinations on a single hydrolyzate afforded a reasonably reliable estimate of the glutamic acid content of the products examined.

Literature Cited

(1) Haugaard, Gotfred, and Kroner, T. D., J. Am. Chem. Soc., 70, 2135 (1948).
(2) McFarren, E. F., Anal. Chem., 23, 168 (1951).
(3) McFarren, E. F., and Mills, J. A., Ibid., 24, 650 (1952).
(4) Olcott, H. S., J. Biol. Chem., 153, 71 (1944).

MOISTURE IN GRAIN

Hygroscopic Equilibria of Rough Rice at Elevated Temperatures

JOSEPH T. HOGAN and
MELVIN L. KARON[1]

Southern Regional Research Laboratory, New Orleans, La.

The hygroscopic equilibria of rough rice have been determined at 80° to 111° F. for moisture contents of 11 to 22%, dry basis. The data have been correlated by an Othmer plot in which temperature, moisture content, and vapor pressure data are presented graphically for use in storage, packaging, processing, and drying. The plot permitted calculation of the relative isosteric heat of adsorption of water by rough rice of varying moisture content. Hygroscopic data were examined by the Harkins-Jura equation, which indicated that adsorption of water occurs as a condensed film over 14 to 22% moisture content. Application of the Brunauer-Emmett-Teller equation to available data on rough rice indicated that adsorption of moisture as a unilayer occurs over a range from dryness to approximately 7% moisture content. The amount of water, which constitutes a unilayer of adsorbed water on rough rice, is approximately 7 grams per 100 grams of dry rough rice and is identical calculated by Harkins-Jura or Brunauer-Emmett-Teller equations. Adsorption of water on rough rice occurs in three stages. The first, 0 to 7% moisture content, represents a unilayer of water molecules; the second is characterized by addition of an equal number of molecules to the already adsorbed unilayer; and the third may be considered a multilayer addition of water from approximately 14% moisture to saturation.

T HE MOISTURE CONTENT of a material in hygroscopic equilibrium depends upon the relative humidity of the surrounding atmosphere but varies widely with different materials. The amount of moisture contained in a hygroscopic material in equilibrium with the surrounding atmosphere is properly defined

[1] Present address, Standard Fruit and Steamship Co., New Orleans, La.

as the hygroscopic moisture; industrially this hygroscopic moisture is frequently termed "moisture regain" and is expressed in parts of water per 100 parts of dry material by weight. Inasmuch as the moisture contents of hygroscopic materials seriously affect the storage and keeping qualities of such materials, equilibrium moisture data are directly applicable to the solution of storage, processing, and drying problems.

Rice, like other grains, is hygroscopic in nature—that is, it gains or loses moisture when the vapor pressure of water in the space surrounding the grain is greater or less than the vapor pressure exerted by the moisture within the grain. This equilibrium moisture content has previously been determined for rice and other grain by exposing samples of the grain to an atmosphere of constant temperature and relative humidity main

tained in sealed desiccators by solutions saturated with salts. Changes in moisture content occur until the vapor pressure of water in the samples equals the vapor pressure of water in the atmosphere maintained by the saturated salt solution. After equilibrium has been attained, the moisture content of the rice is determined by an accepted method and a point on the curve of "equilibrium moisture content vs. relative humidity" is established. A series of observations with different saturated salt solutions, which maintain different relative humidities, provides the desired equilibrium moisture curve.

Coleman and Fellows (5) were the first to investigate the hygroscopic equilibria of rough rice, measuring it at 25° C. Karon and Adams (8) determined hygroscopic equilibria at the same temperature not only for rough rice but also for rice fractions (hulls, undermilled or brown rice, bran, and milled rice). The procedure used by both teams of investigators to obtain the relative humidity of atmosphere in contact with the rice and fractions thereof was that described above—i.e., desiccators and saturated salt solutions at constant temperature. Difficulties experienced with this procedure are recognized. If the air in the desiccator is not stirred, long periods of time are required to reach equilibrium; in the Karon-Adams study approximately 20 to 25 days were necessary to attain equilibrium. Because of the long exposure period required, samples exposed at approximately 85% relative humidity or higher tend to mold before equilibrium is reached. Values of equilibrium moisture content for such samples are usually high, representing in fact an equilibrium moisture content for mold and rice.

At the Rice Technical Committee Meeting at Beaumont, Tex., January 15 and 16, 1953, the Harvesting, Drying and Storage Committee recommended "that more basic information on hygroscopic equilibrium of rough rice be obtained over a wide range of conditions which are encountered during harvest, drying, and storage of rice."

The present study of the hygroscopic moisture relationships of rice was undertaken to supplement published data and to furnish hygroscopic equilibria data over a range of relative humidities at the elevated temperatures of 80° to 115° F. (27° to 46° C.). These data yield, in addition, information on the temperature coefficient of the hygroscopic moisture content of rice and lend themselves to physical chemical interpretations for estimating the heat of adsorption of water by rice. The vapor pressure data obtained in this study are applicable to drying operations.

Materials and Methods

Materials Freshly combined Zenith rice, which had been flown by plane from Crowley, La., to the Southern Regional Research Laboratory, New Orleans, La., in September 1953, was used immediately for the study of hygroscopic equilibria. The rice was spread in shallow wooden trays with wire-screen bottoms, 24 × 36 × 6 inches, and allowed to air-dry in the laboratory. Portions of the rice were removed at intervals, giving samples of approximately 11, 14, 16, 20, and 22% moisture content, dry basis, for equilibration studies.

Dynamic Method for Obtaining Hygroscopic Equilibrium Several investigators have determined the hygroscopic equilibria of rough rice (16, 17) and whole kernel corn (7) by measuring with an electric hygrometer the relative humidity of the interseed air in equilibrium with grain conditioned to selected moisture contents, the grain samples in each case being stored under static conditions in a sealed container, such as a Mason jar. The data resulting from these studies on rough rice remain unpublished and are generally unavailable. In view of the advantages reported for this type of method and because of the difficulties experienced with the desiccator–saturated salt solution method, a dynamic method for

equilibrating rice was tried and found to be satisfactory.

In this procedure rice is sealed in a chamber which permits circulation of the enclosed air through the rice. This chamber consists of a cylindrical metal container, closed at one end and open at the other, and fabricated from No. 22 gage sheet copper, approximately 15 inches high and 8 inches in diameter (Figure 1). The upper open end was provided with a brass flange to which was fitted a $^1/_4$-inch cover plate. Inserted in the cover plate were a centrifugal blower and a manually operated selector bearing eight hygrometer sensing elements. The hygrometer employed in this study was the Aminco-Dunmore electric hygrometer indicator, provided with multiple sensing elements. This equipment permits measurement of relative humidities in the range 2 to 99% with an error of ±1.5% relative humidity, providing the sensing element most suitable for the humidity measured is selected for each reading.

The chamber was fitted with a false bottom made of stainless steel mesh and located approximately 2.5 inches above the bottom of the chamber. The centrifugal blower and the bottom of the chamber beneath the false bottom were connected by a 2-inch copper pipe, rigidly soldered into the cylinder wall beneath the false bottom and attached to the inlet of the blower by means of a flange. The connections between the cover plate and cylinder, and between the copper-pipe flange and blower, were made by means of $^1/_4$-inch hexagonal bolts and nuts.
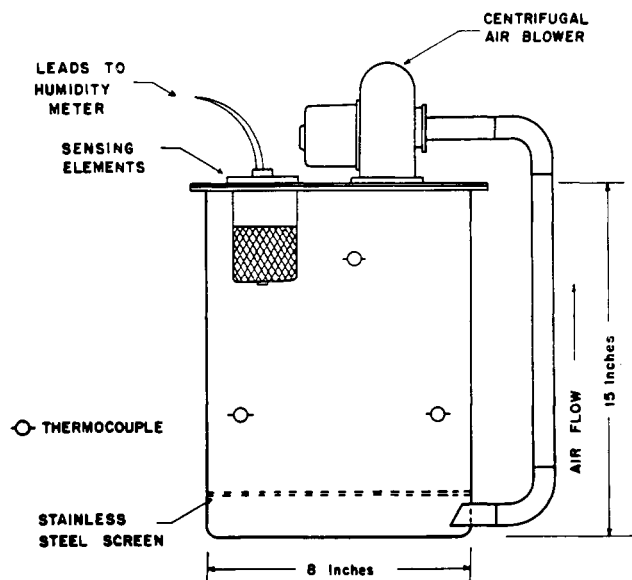
Removal of the cover plate was accomplished by unfastening the bolts and nuts of the cover plate as well as those of the inlet flange to the blower.

The physical dimensions of the chamber are such that the ratio of the volume of air to the weight of rice is kept to a low value, which is established by consideration of the extreme conditions of temperature and relative humidity variation to which the rice will be subjected. Normally during recirculation of the air through the rice, the vapor pressure of the air adjusts itself to that of the rice. There is an exchange of moisture from rice to air, or vice versa, depending upon the initial moisture content of the rice and initial relative humidity of the circulating air. The weight of rice chosen, 8 to 9 pounds, is such that the greatest exchange of moisture anticipated during operation will represent an insignificant change in moisture content of the rice.

Procedure

During operation the chamber was immersed in a constant-temperature water bath, the level of the water being adjusted to within $^1/_2$ inch of the top flange of the container. To hasten heat penetration into the interior of the

Figure 1. Rice equilibrium chamber

LEADS TO HUMIDITY METER

SENSING ELEMENTS

CENTRIFUGAL AIR BLOWER

AIR FLOW 15 Inches

○ THERMOCOUPLE

STAINLESS STEEL SCREEN

8 Inches

chamber, an internal copper coil, helical in shape, was centrally located in the chamber and the water from the constant-temperature water bath was circulated through the coil. Thermocouples were used to record the temperatures of the circulating air and rice bed during the progress of an experiment. During operation the chamber was filled with 8 to 9 pounds of rice of predetermined moisture content and the chamber sealed by fastening the cover plate and inlet flange of the blower securely. The air blower was operated at a speed sufficient to ensure rapid circulation of the inclosed air through the rice bed. A moisture exchange takes place, to permit an equilibrium to be reached wherein the aqueous vapor pressure of the air equals that of the rice. Relative humidity of the circulated air was measured at 15-minute intervals with the electric hygrometer. Equilibrium was judged to have been attained when the relative humidity of the air became constant. After equilibrium of temperature and relative humidity had been virtually attained, the operation was continued for 1 hour longer and a final humidity reading was taken. Approximately 5 hours were required for an experiment at one temperature.

After equilibration at one temperature, the water bath temperature was elevated and the experiment repeated, recordings being made of the relative humidity and temperature as before.

Results

The experimental results obtained by the dynamic method of equilibrating rice are shown in Figure 2. Equilibrium curves for rough rice exhibit the characteristic form, which is typical of the curves for most grains. For purposes of comparison the data obtained at 25° C. (77° F.) on rough rice by Coleman and Fellows (5) and Karon and Adams (8) are also included. The effects of temperature disclose two relationships: (1) The equilibrium moisture content of rough rice at a given relative humidity is higher at lower temperatures—i.e., as the temperature of the grain rises, moisture tends to leave the grain so as to bring the vapor pressure of the surrounding atmosphere into equilibrium with the vapor pressure of the moisture in the rice; (2) as the moisture content of the grain increases in the range 11 to 22% moisture content, dry basis, the effect of temperature on the equilibrium moisture content decreases. The data of Karon and Adams at 77° F. (25° C.) closely parallel the data obtained in this experiment at 80° F.

Correlation of Experimental Data

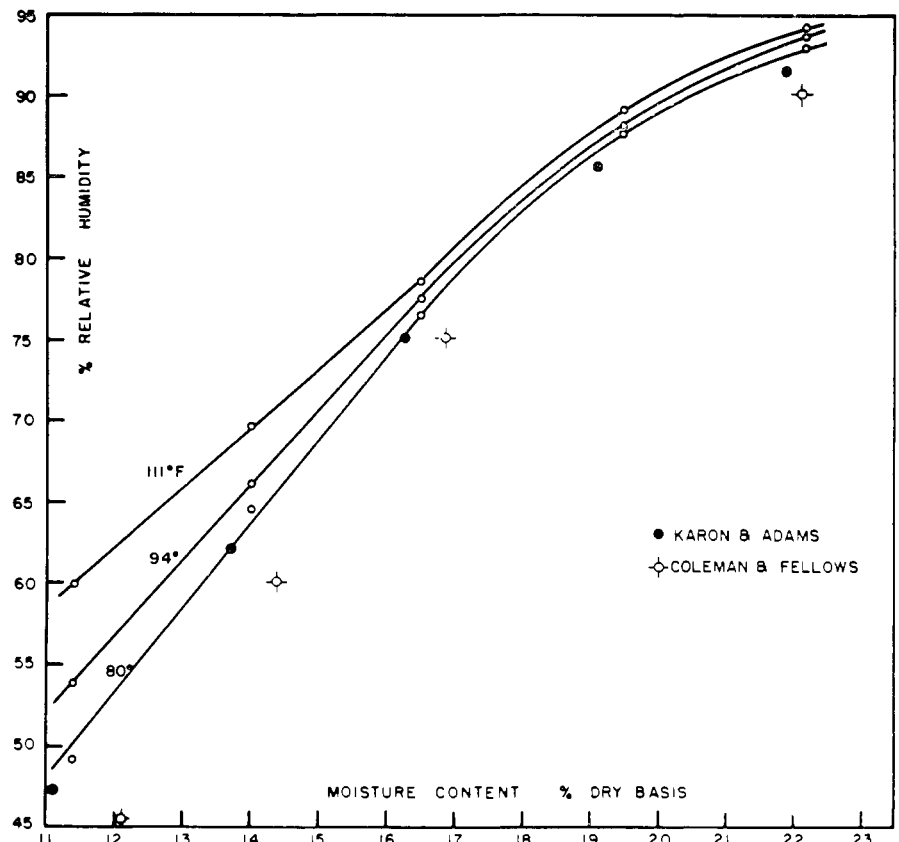Plots of the original data of equilibrium moisture content against relative

Figure 2. Hygroscopic equilibrium curves of rough rice

humidity, as in Figure 2, are inadequate to permit the extrapolation of experimental data beyond the measured range of temperatures of the experiment. For best presenting the data it is desirable to apply a satisfactory correlation, preferably with theoretical basis, for the existing data, thereby eliminating minor inconsistencies and permitting extrapolation to lower or higher temperatures when desired. Three types of mathematical treatments, found useful by other investigators, have been used on rice data in this paper and in the earlier paper of Karon and Adams (8).

Othmer (12–15) developed a new basic relationship between vapor pressures of pure liquids and those of solutions and adsorbents. For materials containing water, Othmer compared the vapor pressure, P, of water over solutions, paper, adsorbents, etc., with the vapor pressure, P_0, of pure water at the same temperatures and found that the plot of log P vs. log P_0 yielded straight lines if the temperature dependencies of the isosteric heat of adsorption of water by the material and the heat of condensation of pure water vapor are similar. It is apparent that lines on such a coordinate plot must represent one moisture condition only, and hence are isosteres. Furthermore, as the saturation vapor pressure of water is uniquely determined by temperature, this method of plotting provides a convenient means of introducing the temperature variable. In addi-

tion, Othmer has shown that the slope of the lines (isosteres) on such a log-log plot is numerically equal to the ratio of the isosteric heat of adsorption of water by the material to the heat of condensation of water vapor at the same temperature.

As a first step in using the Othmer method the data of Table I were ob-

Table I. Relative Humidity of Air in Equilibrium with Rice of Constant Moisture Content

Moisture Content, % Dry Basis	Temperature, ° F.		
	80	94	111
	Relative Humidity, %		
11	48.1	52.1	58.6
12	53.3	56.7	62.2
14	64.5	66.0	69.6
16	74.2	75.2	77.0
18	82.6	83.5	84.4
20	88.9	89.5	90.4
22	92.6	93.4	94.0

tained by reading the values of the relative humidity at integral values of 11, 12, 14, 16, 18, 20, and 22% moisture content from Figure 2. The saturation vapor pressures of water at 80°, 94°, and 111° F. were multiplied by the relative humidity values shown in Table I to furnish the vapor pressures of water in the rice at the values of temperature and moisture content presented in Table II. The logarithms of the vapor pressures
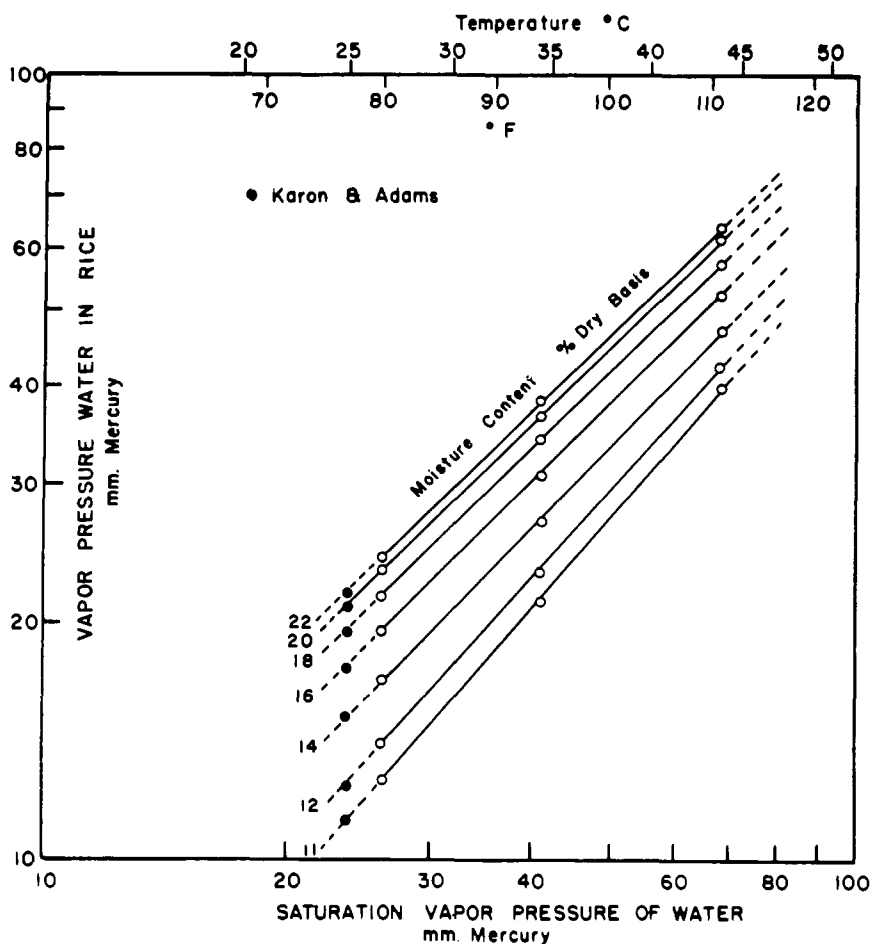
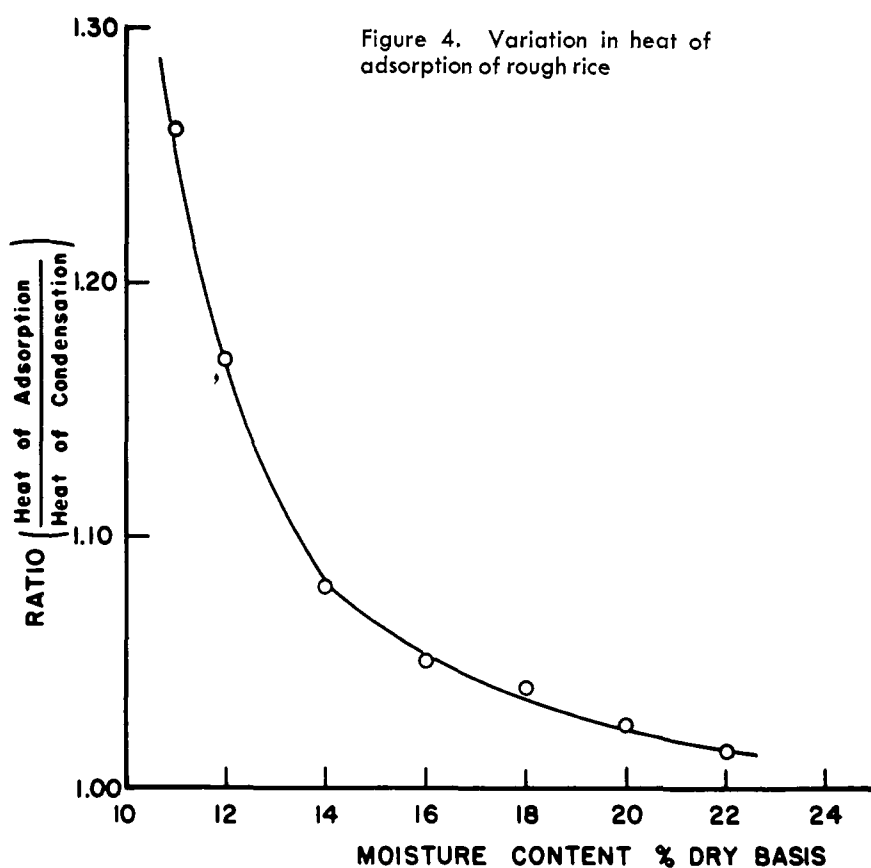Figure 3. Othmer vapor pressure log-log plot

Figure 4. Variation in heat of adsorption of rough rice

of water in rough rice are plotted in Figure 3 against the logarithms of the saturation vapor pressures of pure water at the same temperatures. Each line (isostere) represents a given moisture content. The temperature scale is also indicated at the top of the plot.

The plots shown in Figure 3 yield straight lines for each integral moisture content. Straightness of these individual lines of constant water composition indicates a reasonable verification of the one assumption involved—i.e., in the range of the conditions of the measurements, the temperature coefficient of the isosteric heat of adsorption of water on rough rice is similar to the temperature coefficient of the heat of condensation of water vapor.

Table II. Aqueous Vapor Pressures of Equilibrated Rice

| Moisture Content, % Dry Basis | Temperature, ° F. | | |
| | 80 | 94 | 111 |
	Vapor Pressure, Mm.		
11	12.6	21.3	39.8
12	14.0	23.2	42.3
14	16.9	27.0	47.3
16	19.5	30.8	52.3
18	21.6	34.2	57.3
20	23.3	36.6	61.4
22	24.2	38.2	63.8

The slopes of these lines increase as the moisture content decreases. This indicates that as the rice becomes drier the heat of adsorption of a unit weight of water increases. The ratio of heat of adsorption of water by rough rice to the heat of condensation of water vapor at the same temperature is equal to the slopes of the isosteres in Figure 3. These ratios for rice of varying moisture contents are shown graphically in Figure 4. The value of the ratio increases slowly from 1.01 for rice of 22% moisture content to 1.08 for rice of 14% moisture content, and then increases very sharply to a value of 1.26 at 11% moisture. Thus, the heat of adsorption of water by rough rice that contains 11% water is approximately 1.3 times the heat of condensation of water at the same temperature.

In an attempt to analyze the water adsorption data of rough rice in somewhat greater detail, the theory of Harkins and Jura (7) has been applied to the present study. The Harkins-Jura equation is based on the assumption that the adsorbed molecules of water form a condensed film. It may be represented in the form

$$\text{Log } (P/P_0) = B - A/V^2$$

where P/P_0 is the relative vapor pressure of the adsorbed water and V is the weight of water adsorbed by 1 gram of the solid. If log (P/P_0) is plotted

against the reciprocal of the square of the amount of water adsorbed, a linear relation is exhibited where the adsorbed film is condensed. This plot of the data in Table I is shown in Figure 5. In general, the plot of log (P/P_0) against $1/V^2$ yields lines that are nearly straight between the values of 20 and 50 for $1/V^2$. Above the value of 50 for $1/V^2$ deviation from linearity occurred, indicating that the adsorption of water vapor on rough rice up to this part of the isotherm obeys the Harkins-Jura theory very well and that the adsorbed water occurs as a condensed film. Rice having a value for $1/V^2$ of 50 contains approximately 14% moisture and is in equilibrium with an atmosphere having a relative humidity of approximately 66%. Reference to Figure 4 indicates that the heat of adsorption of water by rough rice at approximately 14% moisture content or lower increases very sharply at this point. This is interpreted to mean that at approximately 14% moisture content and lower the mechanism of water adsorption on rough rice differs from that at higher moisture contents.

Brunauer, Emmett, and Teller (2, 3) extended the Langmuir treatment of unilayer gaseous adsorption to cover the case where more than one layer of molecules are adsorbed on a solid. It was found that when $P/a(P_0 - P)$ is plotted against P/P_0, a straight line is obtained whose intercept on the y axis is $1/a_1c$ and whose slope is $(c - 1)/a_1c$, where a is the grams of water adsorbed at a vapor pressure of P, and P_0 is the vapor pressure of pure water; a_1 is the grams of water adsorbed in the first layer on the surface of 100 grams of dry solid; c is related to the heat of adsorption. The Brunauer-Emmett-Teller equation has been found to be valid for the range of relative vapor pressures of approximately 0.05 to 0.5. A plot of the data of Karon and Adams (8) for rough rice at 77° F. (25° C.) is presented in Figure 6. The value of a_1 has been calculated to be 7.2 grams of water per 100 grams of dry rough rice.

Liang (9) has shown that the slope, A, of the linear Harkins-Jura plot is equal to a_1^2. Thus, the square root of the slope of the isotherms on the Harkins-Jura plot yields values of a_1. This value of a_1 has been calculated from the plots in Figure 5 and found to be equal to approximately 7.1 grams of water per 100 grams of dry rough rice at 80° and 94° F. The quantity of water in the unilayer as calculated by the Harkins-Jura equation is the same as that found by the Brunauer-Emmett-Teller equation.

Dunford and Morrison (6) have postulated that this identical value of a unilayer for the upper and lower parts of the isotherms may be explained by the formation of a film at least two molecules thick, the upper layer be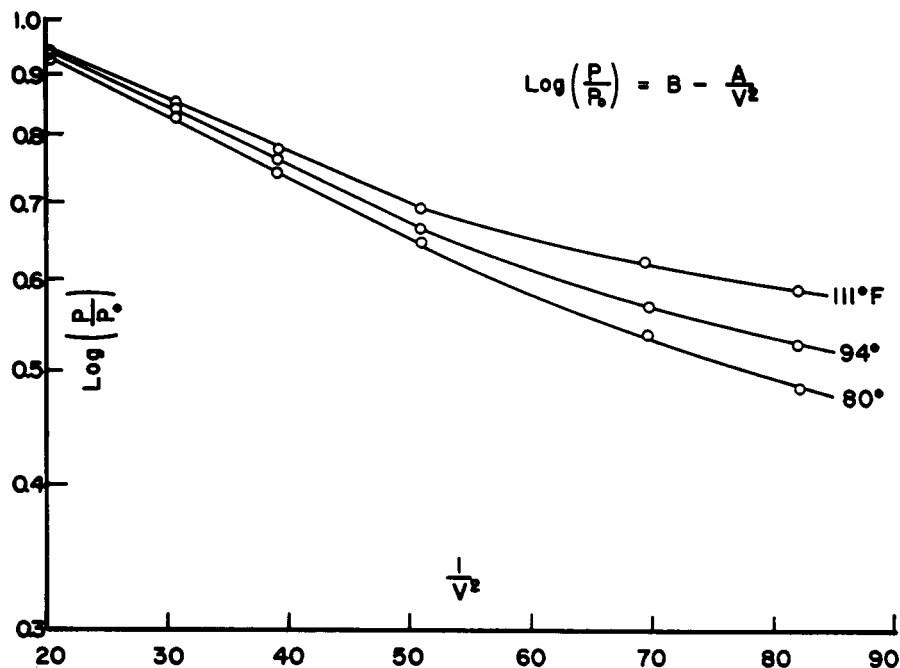ing condensed and occupying the same surface area on the underlying unilayer. Their observations are further substantiated by the fact that in all cases a weight of water greater than a_1 is adsorbed before a relative vapor pressure of 0.5 is reached. They concluded that this identity of values of the upper and lower branches of the isotherm signifies that the same number of adsorption sites are indicated. Assuming that the same number of adsorption sites are involved in the adsorption of the second layer of water, one can expect the completion of the second layer to occur at a value approximately equal to 2 × 7.1. This value of the quantity a_2, the water adsorbed upon completion of the second layer of water, was obtained by plotting $a/(P/P_0)$ against P/P_0 and selecting the point at which $a/(P/P_0)$ reaches a minimum (4). This value is found to be approximately 14% moisture content or approximately 2 × 7.1, the value of a_1, the amount of water adsorbed on a unilayer according to the Brunauer-Emmett-Teller theory.

The process of water adsorption from

Figure 5. Harkins-Jura plot for rough rice

$$\text{Log}\left(\frac{P}{P_0}\right) = B - \frac{A}{V^2}$$

Figure 6. Brunauer-Emmett-Teller plot for rough rice

$$\frac{P}{a(P_0-P)} = \frac{1}{a_1 c} + \frac{c-1}{a_1 c} \cdot \frac{P}{P_0}$$
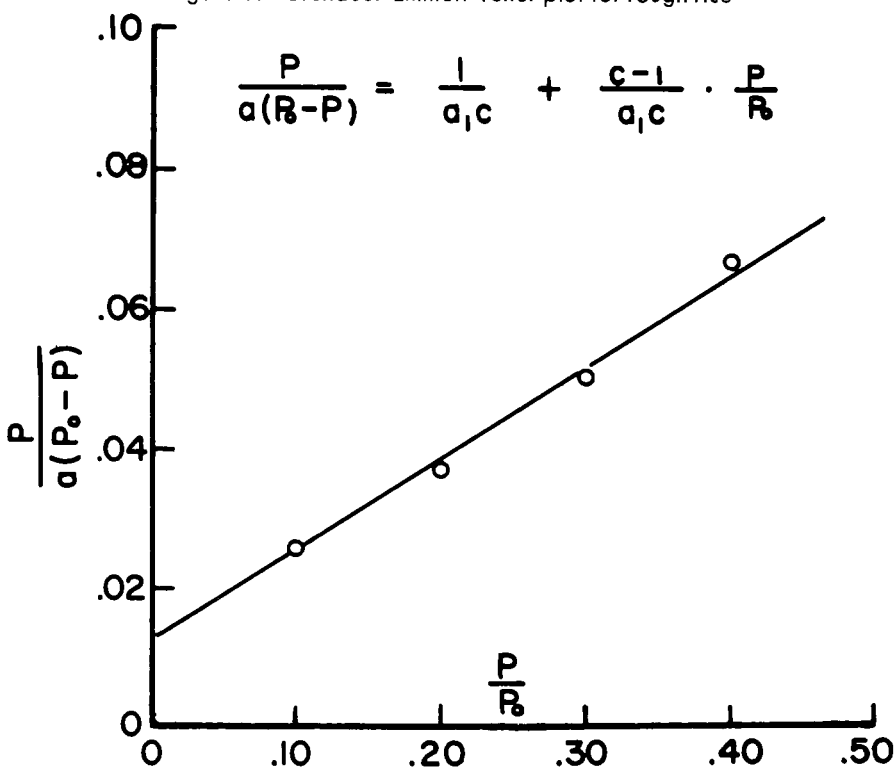
dryness to saturation appears to fall into three stages, thus giving rise to the sigmoid-type curve when relative humidity is plotted against moisture content. For rough rice the first stage extends from dryness to approximately 7% moisture content (30% relative humidity) and this point is characterized 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.

Literature Cited

(1) Brockington, S. F., Dorin, H. C., and Howerton, H. K., *Cereal Chem.*, **26**, 166 (1949).
(2) Brunauer, S.. "Adsorption of Gases and Vapors," Vol. **I**, p. 161, Princeton University Press, Princeton, N. J., 1943.
(3) Brunauer, S., Emmett, P. H., and Teller, E., *J. Am. Chem. Soc.*, **60**, 309 (1938).
(4) Bull, H. B., *Ibid.*, **66**, 1499 (1944).
(5) Coleman, D. A., and Fellows, H. C., *Cereal Chem.*, **2**, 275 (1925).
(6) Dunford, H. B., and Morrison, J. L., *Can. J. Chem.*, **32**, 558 (1954).
(7) Harkins, W. D., and Jura, G., *J. Am. Chem. Soc.*, **66**, 1366 (1944).
(8) Karon, M. L., and Adams, M. E., *Cereal Chem.*, **26**, 1 (1949).
(9) Liang, S. C., *J. Phys. & Colloid Chem.*, **55**, 1410 (1951).
(10) Makower, B., *Ind. Eng. Chem.*, **37**, 1018 (1945).
(11) Makower, B., and Dehority, G. L., *Ibid.*, **35**, 193 (1943).
(12) Othmer, D. F., *Ibid.*, **32**, 841 (1940).
(13) *Ibid.*, **34**, 1072 (1942).
(14) Othmer, D. F., and Sawyer, F. G., *Ibid.*, **35**, 1269 (1943).
(15) Othmer, D. F., and White, R. E., *Ibid.*, **34**, 952 (1942).
(16) Todd, M. L., Todd, Hedeen and Associates, Waterloo, Iowa, personal communication.
(17) Wratten, F. T., U. S. Dept. Agr., Agr. Research Service, Louisiana State University, Baton Rouge, La., personal communication.

SPICE ANALYSIS

Spectrophotometric Method for Determining Piperine in Oleoresins of Black Pepper

HAROLD J. FAGEN, EUGENE P. KOLEN, and RALPH V. HUSSONG

Kraft Foods Co. Research Laboratories, Glenview, Ill.

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