al showing color interference was cocoa (dark brown hydrolysate). Because of slight differences in the pump rates and dialysis membranes associated with a particular channel, the two channels were seldom perfectly balanced. However, it was shown by sampling a solution of FD&C yellow no. 6 and recording the absorbance increase with and without a reference flowcell that between 80 and 100% of the color remaining after dialysis was removed by splitting the sample and employing a reference channel. This amount of imbalance proved to have a negligible effect. The effectiveness of dialysis and the reference flowcell were evidenced by the lack of color interference observed with the large number of products used in this study.

The color formation at 470 nm is a function of the pH of the solution. Since the food products studied differed in the degrees of acidity and the amount of fat present, it was of interest to determine if the buffer added after hydrolysis was sufficient to change the pH to that of the standards. By varying the pH 0.5 units from the standard, a maximum error of 2% was encountered. Of the 63 different food products studied, the maximum pH difference, after addition of sample buffer, between hydrolysate and standard was 0.3 units; thus, it was demonstrated that the pH difference between hydrolysate and standards produced no significant error.

Recovery studies were conducted with niacinamide to determine if the hydrolysis conditions were severe enough to quantitatively hydrolyze the amide and extract the niacin from the food product. The products were determined for their niacin content prior to addition of the niacinamide solution. The mixtures were carried through the procedure described in the Experimental Section and the results are summarized in Table I.

Throughout this study, excellent linearity was observed for the standard solutions of nicotinic acid.

The automated method was compared to the microbiological method because the latter is specific for niacin and is sensitive. In this study, five replicates of each food product or ingredient were analyzed with the automated method and the results were compared to those obtained with a microbiological assay (Association of Official Analytical Chemists, 1970) which employed severe acid hydrolysis. The results of the comparison between the two methods are shown in Table II. A 0.9937 correlation coefficient and a standard error of 1.2 mg/100 g were observed between the two methods. The excellent precision of the automated method was evidenced by an average relative standard deviation of only 1.5%, which reflects all errors incurred in sampling, extraction, and analysis. The long-term reproducibility was demonstrated by three different products (RTE (ready-to-eat) cereal product 1, snack 2, and cake mix 6) which were analyzed three times at 1 week intervals. The results of this study demonstrate that the automated procedure is the method of choice for the determination of niacin and niacinamide in a wide variety of food products. By hydrolyzing large groups of samples, an analysis rate can be achieved that cannot be approached by a microbiological or manual chemical method.

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Technique for Measurement of Water Activity in the High A_w Range

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A method was developed to determine the water activity (A_w) of food systems in the range of 0.85 to 0.98 A_w. The method is based on the equilibrium moisture absorption of microcrystalline cellulose at a given temperature. Sulfuric acid solutions of known concentration and A_w were used to prepare a standard curve of equilibrium moisture absorption *vs.* A_w at 35°. A known amount of the standard microcrystalline cellulose was placed in desiccators containing about 50- to 100-g food sample and evacuated for 1.5 min. After 24 hr, the weight gain of the cellulose was measured and the moisture content calculated. Results show that the method is comparable to that of the electric hygrometer and considerably better than the manometric technique.

The control of water activity (A_w) in the processing of foods is of major importance in relation to microbial spoilage and growth of pathogens. It is only at the higher A_w range, 0.90 to 0.99, that microorganisms usually grow in foods, and the rate of growth of most microorganisms is greatly accelerated at the higher A_w's.

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In this high range of A_w's, measurement by the electric hygrometer of A_w based on the electrical resistance of a salt-coated probe is inaccurate and sometimes misleading (Troller, 1973). Hygrometer probes are accurate to within ±0.005 A_w when new, but with age become less accurate so they must be recalibrated constantly. They are also subject to errors due to absorption of volatiles, such as glycerol, from the food (Block *et al.*, 1961). Measurement by a manometric technique as described by Labuza (1974) based on the design of Karel and Nickerson (1964) of the

Table I. A_w of Food Samples Obtained by Three Methods

Method	Bread	Salami	Whey concentrate	Pancake batter[a]
Microcrystalline cellulose method	0.951	0.969	0.820	0.961
	0.948	0.968	0.821	0.960
	0.948	0.969	0.820	0.960
Av	0.949	0.969	0.820	0.960
Manometric technique	0.94	1.00	0.89	0.97
	0.92	0.98	0.87	1.00
Av	0.93	0.99	0.88	0.99
Hygrometer	0.945	0.970	0.805	0.895
	0.955	0.965	0.820	0.871
Av	0.950	0.968	0.815	0.883

[a] One-month old probe used.

vapor space surrounding the food is also inaccurate at A_w's greater than 0.90 because of temperature control problems. An accuracy of ±0.005 below 0.85 is expected; above that, it falls to ±0.02. Also, if temperature is not controlled accurately, condensation of water vapor occurs and the results become meaningless.

Fett (1973) devised a method to measure A_w greater than 0.80 in foods based on the equilibrium moisture absorption of standard proteins at a known A_w and a given temperature. At A_w's greater than 0.95, more than 24 hr was necessary to achieve moisture absorption equilibrium. Also, a standard curve had to be made for each new batch of protein.

The present study was conducted to devise a simple technique that would give an accurate measurement of A_w's greater than 0.90 within 24 hr, based on the equilibrium moisture absorption of microcrystalline cellulose at a given temperature. In addition, cellulose would be better to use since proteins oxidize with age and change their absorption slightly whereas the crystalline cellulose is extremely stable (Bluestein and Labuza, 1972). In their work, they showed the same B.E.T. monolayer for the cellulose as was found by Maloney *et al.* (1966) working six years previously.

EXPERIMENTAL SECTION

The A_w of several food products was measured using three methods: (1) Hygrodynamics electric hygrometer (model 15-3001); (2) manometric technique; (3) equilibrium moisture absorption of microcrystalline cellulose (Avicel FMC Corp., Marcus Hook, Pa.). For measurement by the hygrometer, approximately 5 to 10 g of the food was placed in jars which contain the sensor in the cover. They were allowed to equilibrate at 35° for 24 hr prior to reading. The same amount of food was used for A_w measurement by the manometric device described by Labuza (1974). For the new method, the microcrystalline cellulose was dried in a vacuum oven for 48 hr, 100°, 29 mm Hg. Samples of 2 g (to 0.0001 g) of the standard dried microcrystalline cellulose were weighed into 35-ml weighing bottles. The cap was removed from the weighing bottle and triplicate samples were placed on the plate in a 214.9-cm vacuum desiccator containing 50 to 100 g of the food sample. The desiccators were evacuated for 1.5 min and were placed at 35° for 24 hr. No measurable loss of water occurs in this evacuation time. After 24 hr, air was gradually let into the desiccators over a period of 5 min (at this high temperature, there was no condensation apparent). The weighing bottles were capped and wiped dry prior to weighing. The moisture content was calculated from the weight gain.

The A_w of the food product was determined by referring to the standard curve (Figure 1) in which A_w is plotted *vs.* moisture content for the microcrystalline cellulose at equilibrium. This isotherm was found by measuring the adsorption isotherm of Avicel over standard sulfuric acid–water solutions (Wilson, 1940). The exact composition of the sulfuric acid solutions was determined by titration with base. Quadruplicate samples were used in preparation of the curve. The accuracy is about ±0.002 A_w as has been found by Fett (1973).

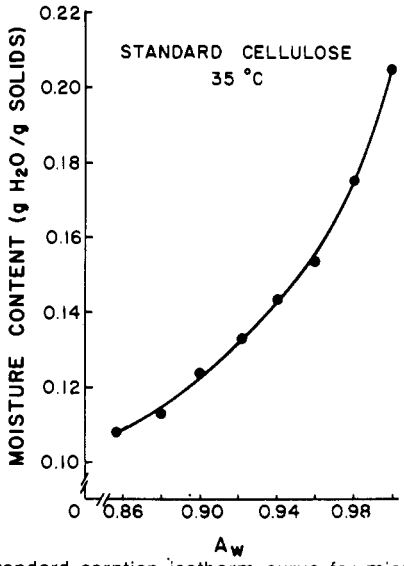

Figure 1. Standard sorption isotherm curve for microcrystalline cellulose at 35°.

RESULTS AND DISCUSSION

A comparison of results obtained by the 3 methods (Table I) demonstrates that the microcrystalline cellulose method is comparable to the measurements using a hygrometer with a new sensor. The manometric technique gave results very different from the other two methods. This was due to the difficulty in preventing condensation at the high A_w.

The microcrystalline cellulose method may be used for measurement of A_w's greater than 0.90 and should be more accurate, especially if new hygrometer probes are not available. This method is more valuable than the use of the hygrometer or manometric device since it does not involve the expense of a special instrument. The hygrometer probes lose their accuracy with time, as is seen in the data for the pancake batter. The probe used was 1-month old, and was recalibrated over saturated sodium chloride but still gave a different value than the cellulose method. This could be due to the volatiles in the batter causing interference. The larger food sample size (about 10 times that used in other techniques) is also advantageous in that a more representative sample of the food product may be used for the A_w measurement.

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