also be useful in commerce to distinguish high-lysine lines of corn, which may exhibit diverse physical and compositional differences from low-lysine types. Modifications are being tested to determine whether the accuracy of this method can be improved to permit analysis of small differences in lysine within a class of corn and whether the standard curve can be extended to include samples containing higher protein and higher lysine levels.

ACKNOWLEDGMENTS

The following supplied corn samples: A. V. Paez, Pioneer Hi-Bred Corn; D. Storck, P-A-G; C. Lable, Funk Bros. Seed Co.; V. Ferguson, Bear Hybrid Corn Co.; F. D. Poey, Poey Hi-Bred Seed Co., Mexico; E. Villegas, International Maize and Wheat Improvement Center, Mexico; J. W. Nordstrom, University of Minnesota; L. F. Bauman, Purdue University; and D. E. Alexander, University of Illinois. Miss I. M. Cull provided the endosperm samples obtained by hand dissection of whole grain.

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Received for review September 24, 1973. Accepted December 3, 1973. Presented at the 166th National Meeting of the American Chemical Society, Chicago, Ill., August 26-31, 1973. Mention of firm names or trade products does not imply that they are endorsed or recommended by the Department of Agriculture over other firms or similar products not mentioned.

The Semiautomated Determination of Niacin and Niacinamide in Food Products

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A general continuous flow scheme has been developed which employs an in-phase reference flowcell to eliminate blank color interference. The flow scheme was used for automating a colorimetric determination of niacin in a wide variety of food products. A simplified sample preparation was developed which yielded recoveries ranging from 94 to 100%. The semiautomated procedure was shown to compare favorably with a specific microbiological assay for 63 different products (0.9937 correlation coefficient). The average relative standard deviation of the automated method was 1.5% for the 63 products, with niacin levels ranging from 0.83 to 54.7 mg/100 g. The main advantage of this procedure is the large number of analyses which can be rapidly effected. Fifty samples per hour can be analyzed after sample preparation.

The development of more rapid and accurate methods for determining the nutritional quality of food products is becoming increasingly more important. The determination of vitamins such as niacin is a prime consideration. Because of the inherent difficulties with chemical methods, microbiological assays are often used for this vitamin (Association of Vitamin Chemists, 1947; Strohecker and Henning, 1965). Although these assays are specific and sensitive, they are tedious, time consuming, and lack desirable reproducibility.

Studies have been conducted comparing manual chemical assays with microbiological methods (Melnick, 1942; Steele, 1945). While some workers have found good agreement, other workers have observed wide discrepancies. Gorin and Schütz (1970) observed good correlation between a microbiological method and a spectrophotometric method after thin-layer chromatography cleanup.

Colorimetric methods for the determination of nicotinic acid are based on the König reaction (König, 1904) of pyridine derivatives with cyanogen bromide and an aromatic amine to form polymethine dyes. It has been reported that the colorimetric method suffers from poor reproducibility due to color instability and pH sensitivity and that the method is tedious and subject to color interference when analyzing natural products (Association of Vitamin Chemists, 1947; Strohecker and Henning, 1965).

In view of the inherent reproducibility and ease of operation of continuous flow systems, it was felt that these problems could be eliminated. An automated niacin method applicable to natural products has been reported (Technicon Instrument Company, 1972); however, the sample preparation is time consuming, a reference flowcell to eliminate interference is not used, and the accuracy of the method has not been established. In the

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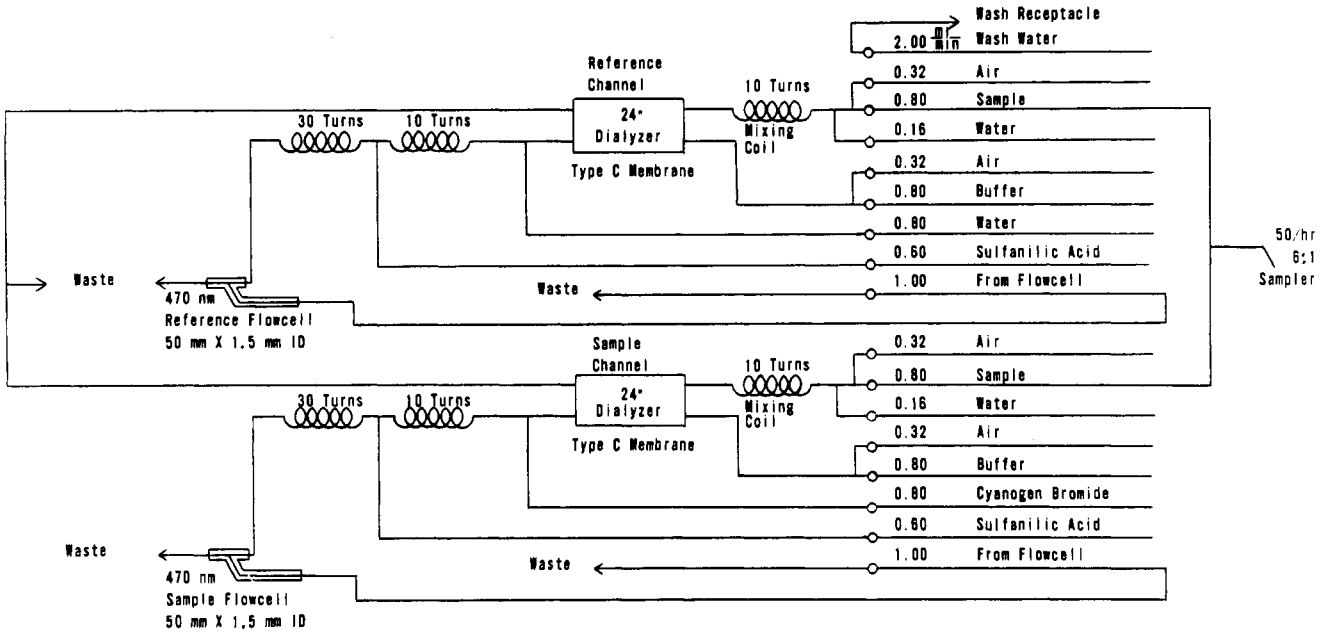

Figure 1. Flow scheme used for the determination of niacin. Flowcells are mounted in a Technicon double beam colorimeter. The waste receptacle contained a concentrated solution of sodium hydroxide for the destruction of excess cyanogen bromide.

present paper we describe a general flow scheme employing a reference flowcell which is applicable for the determination of niacin and for other colorimetric methods. A rapid sample preparation was used and the results are compared with microbiological assay to help define the accuracy of the method.

EXPERIMENTAL SECTION

Apparatus. The dual flowcell flow scheme shown in Figure 1 was constructed from standard Technicon AutoAnalyzer™ II components. The scheme was designed so that chemistries could be easily changed by simply changing reagents, pump tubes, and wavelength for detection.

Reagents. All reagents and solvents were analytical grade. A stock phosphate buffer was prepared by diluting a solution of disodium phosphate (130 g) and potassium dihydrogen phosphate (40 g) to 1 l. with distilled water. The working buffer (pH 7.3) was prepared by diluting 150 ml of stock buffer and 1.5 ml of Brij 35 (35% aqueous, surfactant (Atlas Chemical)) solution to 1 l. with distilled water. The sample buffer (pH 7.6) was prepared by diluting a solution of disodium phosphate (136 g) and potassium dihydrogen phosphate (24 g) to 1 l. with distilled water. Excess monohydrogen phosphate (0.405 F.W./l.) was added to the sample buffer to compensate for the portion which precipitated as calcium salt when the buffer was added to the sample hydrolysate. A 10% sulfanilic acid solution and a 10% cyanogen bromide (very poisonous) solution were prepared using the method previously described (Technicon Instrument Company, 1972). These solutions were not stored for longer than 2 days and were filtered daily or if excessive recorder noise was observed. If bubbles formed in the lines, the cyanogen bromide solution was degassed by drawing a vacuum for a few minutes with a water aspirator mounted in a hood. The problems encountered when handling cyanogen bromide, a common criticism of the chemical niacin method, are drastically reduced when the procedure is automated. The wash solution was prepared by diluting 1.5 ml of Brij 35 solution (35%) to 1 l. with distilled water. Reagent grade nicotinic acid was used to prepare standards fresh each day (0.5 to 5.0 μg/ml).

Procedure. A portion of ground sample containing about 0.1 to 0.4 mg of niacin was added to a 100-ml volumetric flask containing 0.55 g of calcium hydroxide. Approximately 50 ml of distilled water was added and the flask was covered with foil and autoclaved (15 psi, 120°) for 2 hr. (Products containing a high oil content were found to spatter during autoclaving. These products were hydrolyzed in a disposable plastic container and transferred to a volumetric flask after hydrolysis.) After the samples cooled to room temperatures, hydrochloric acid (10 ml, 1.5 *N*) was added to dissolve the remaining calcium hydroxide. A 25-ml portion of sample phosphate buffer was added (precipitate forms) to adjust the pH to approximately 6.7 and the mixture was diluted to volume with distilled water. Two drops of Brij 35 solution were added and the mixture was shaken vigorously and filtered (Whatman no. 1). Standards for calibration were treated exactly as described above.

The instrument was phased (sample and reference reach their respective flowcell simultaneously) by sampling a solution (0.20 mg/ml) of FD&C yellow no. 6 (disodium salt of 1-*p*-sulfophenylazo-2-naphthol-6-sulfonic acid) and adding tubing to the channel stream which reached the flowcell early. The set of nicotinic acid standards (0.5–5 μg/ml) was pumped through the system to establish the standard curve. Excellent linearity was observed over this range.

Table I. Recovery of Niacinamide from Food Products

Product	Sample size, g	Niacin content, μg	Niacinamide added, μg	Recovery,[a] %
Dried potato	2.0	337	100	95
Beef bouillon	0.5	125	100	99
Textured soy	0.5	130	200	96
Corn RTE cereal	0.3	104	200	98
Oat RTE cereal	0.5	93	200	95
Soy breakfast snack	2.0	150	100	100
Pancake mix	2.0	49	141	94
Corn RTE cereal	0.5	87	200	98
Wheat RTE cereal	0.5	106	200	98
Buckwheat RTE cereal	0.3	164	200	100
Biscuit mix	1.0	26	100	98

[a] Average of three replicates.

Table II. Comparison of the Automated Method with the Microbiological Method

Product	Automated method[a] (mg/100 g) ± std dev	Rel std dev, %	Microbio. method, 100 g
Ready-to-eat cereals			
1a. Corn	17.6 ± 0.20	1.1	
1b. Corn	17.3 ± 0.16	0.9	15.8[b]
1c. Corn	17.3 ± 0.11	0.6	
2. Buckwheat	54.7 ± 0.56	1.0	52.9
3. Wheat	21.3 ± 0.12	0.6	18.3
4. Corn	34.8 ± 0.10	0.3	36.7
5. Oat	18.5 ± 0.09	0.5	19.5
6. Oat	23.1 ± 0.12	0.5	21.2
7. Corn	14.5 ± 0.21	1.4	13.4
8. Soy	37.6 ± 0.38	0.9	36.0
9. Corn	47.3 ± 0.64	1.4	50.1
10. Corn	15.8 ± 0.16	1.0	15.2
11. Oat	16.0 ± 0.06	0.4	15.2
12. Oat	17.3 ± 0.06	0.3	13.4
13. Corn	15.0 ± 0.17	1.1	14.5
14. Corn	17.2 ± 0.08	0.4	17.3
15. Oat	13.9 ± 0.06	0.4	14.8
16. Corn	18.8 ± 0.08	0.4	17.3
17. Corn	8.42 ± 0.03	0.4	9.2[b]
18. Corn	11.3 ± 0.06	0.5	11.9[b]
Snacks			
1. Corn	5.73 ± 0.05	0.9	5.9[b]
2a. Corn	5.45 ± 0.06	1.1	5.4[b]
2b. Corn	5.49 ± 0.05	0.9	6.7[b]
2c. Corn	5.43 ± 0.03	0.6	6.8[b]
3. Corn	3.82 ± 0.12	3.1	3.9[b]
4. Corn	9.13 ± 0.10	1.1	7.5
5. Potato	4.10 ± 0.06	1.5	4.4[b]
6. Potato	3.67 ± 0.03	0.8	4.2[b]
Casserole products			
1. Rice	6.03 ± 0.12	2.0	6.2
2. Noodle	6.68 ± 0.08	1.2	7.3[b]
3. Noodle	6.43 ± 0.16	2.5	6.9
4. Potato	4.49 ± 0.05	1.1	5.4
5. Potato	3.30 ± 0.08	2.5	5.1
6. Potato	4.66 ± 0.0	0	5.6
7. Noodle	7.27 ± 0.06	0.8	6.2
Soy breakfast snack			
1. Orange	9.40 ± 0.13	1.4	9.8[b]
2. Cherry	10.3 ± 0.16	1.6	10.4[b]
3. Chocolate	9.47 ± 0.17	1.8	8.2
4. Cinnamon	10.4 ± 0.17	1.6	10.4[b]
5. Butter pecan	7.50 ± 0.11	1.5	9.04
Textured soy products			
1. Chicken	16.8 ± 0.15	0.9	14.7[b]
2. Beef	16.5 ± 0.05	0.3	15.2[b]
3. Ham	17.9 ± 1.09	6.1	15.1
4. Beef	26.1 ± 0.37	1.4	28.0
5. Pork	8.39 ± 0.29	3.5	7.2
6. Bacon	3.10 ± 0.05	1.6	4.0[b]
Mixes			
1. Pancake	2.45 ± 0.07	2.8	2.3
2. Biscuit	2.60 ± 0.05	1.9	2.6
3. Cherry cake	1.70 ± 0.07	4.1	1.7
4. Chocolate cake	1.60 ± 0.04	2.5	1.6
5. Ginger cake	1.40 ± 0.02	1.4	1.5
6a. Walnut cake	1.64 ± 0.04	2.4	1.4[b]
6b. Walnut cake	1.50 ± 0.02	1.3	2.1[b]
6c. Walnut cake	1.70 ± 0.06	3.5	2.3[b]
Potato products			
1. Hash browns	5.89 ± 0.42	7.1	6.3
2. Casserole	4.30 ± 0.06	1.4	4.5[b]
3. Instant potatoes	16.9 ± 0.17	1.0	17.1
Pudding and frostings			
1. Vanilla pudding	3.22 ± 0.05	1.6	3.54
2. Chocolate pudding	3.10 ± 0.05	1.6	3.48
3. RTS chocolate frosting	0	0	0.10
Hot cereal			
1. Wheat base	10.4 ± 0.28	2.7	10.7[b]
Beverage concentrates			
1. Beef	25.0 ± 0.58	2.3	25.0
2. Orange	7.60 ± 0.17	2.2	7.7
3. Apple	6.93 ± 0.13	1.9	6.8
Ingredients			
1. Macaroni noodles	9.06 ± 0.13	1.4	9.0
2. Bleached wheat flour	4.06 ± 0.03	0.7	4.5[b]
3. Oats	0.83 ± 0	0	0.6[b]
4. Cocoa	2.17 ± 0.07	3.2	2.9
5. Hard red wheat	6.16 ± 0.11	1.8	5.8[b]

[a] Average of five replicates. [b] Average of two replicates; the remainder are singlet determinations.

The hydrolysate filtrate was aspirated, split, and dialyzed (see Figure 1). The dialyzed material passed into a recipient stream of working phosphate buffer to which was added a 10% solution of cyanogen bromide followed by a 10% solution of sulfanilic acid. The color formation was continuously measured against the sample blank and the absorbance recorded and compared with the standards.

RESULTS AND DISCUSSION

The flow scheme shown in Figure 1 is general and in essence automates the following steps: (1) sampling an aliquot and diluting; (2) purifying the mixture by dialysis; (3) adding a reagent and mixing, followed by adding another reagent and mixing; (4) allowing a specific reaction time and measuring the change in absorbance against a sample blank.

Because this is the general program for many colorimetric methods, the scheme could potentially be adapted to different determinations by simply changing the reagents, pump tubes, and analysis wavelength. This approach obviates the necessity of changing and aligning flowcells and changing flow schemes when going from one chemistry to another. A relatively long flowcell (50 mm) is used to give the necessary sensitivity where required. If flowcell linearity is exceeded, a decrease in absorbance can be achieved by increasing dilution at the point where the final reagent is added. If a particular determination does not require all the reagents that this flow scheme allows, the addition can be omitted by simply pumping a suitable amount of diluent at that point. By changing reagents and pump tubes, this general flow scheme has been used for both the determination of ascorbic acid (indophenol method) and niacin in our laboratory.

The amide of niacin is biologically active and several N-substituted niacinamides have been shown to be active (Woolley *et al.*, 1938). Therefore, in natural products, niacin bound to other material must be released by hydrolysis with acid or alkali to determine the total niacin content. Enzymes have been used but the recovery is lower (Melnick, 1942). The hydrolysate, after calcium hydroxide hydrolysis, was less colored than after hydrolysis with either 1 *N* sulfuric acid or 0.1 *N* sodium hydroxide and color interference was not a problem. A large portion of the interfering color was removed by dialysis and any remaining interference was cancelled by the reference flowcell. Color interference was determined by aspirating the hydrolysate and pumping it through the system without adding cyanogen bromide. Of the 63 products studied, the only materi-

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.

ACKNOWLEDGMENT

The authors thank Lyman Burns and Glynn Peterson for conducting the microbiological assays.

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Received for review July 9, 1973. Accepted October 23, 1973.

Technique for Measurement of Water Activity in the High A_w Range

Patricia T. Vos and Theodore P. Labuza*

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