QUICK METHOD FOR RIBOFLAVIN IN A HIGH-POTENCY CEREAL PRODUCT[1]

R. H. ANDERSON AND D. F. MORAN[2]

ABSTRACT

For control purposes a quick method for the determination of the amount of riboflavin added to a high-potency ready-to-eat cereal was desired. It was found that mixing of appropriate amounts of samples of the cereal with 10% acetic acid solution in a Waring Blendor or a mechanical shaker for 15 minutes resulted in complete extraction of the added riboflavin. Addition of methanol to extracts resulted in readily filterable mixtures which gave clear, colorless filtrates. Fluorophotometric analyses of the filtrates were in good agreement with amounts of riboflavin added and with analyses of the same samples by the A.O.A.C. method by Merck & Co., Inc., Rahway, N. J.

Routine control of processed foods requires rapid analytical methods in order to minimize rejection of product that does not meet specifications.

The A.O.A.C. methods (1) regularly used to determine riboflavin are time-consuming and complicated for the following reasons.

1. Riboflavin in natural materials is present as a part of a complex aggregate with other constituents, and can be extracted only after prior autoclaving with acid or by enzymatic digestion.

2. Hydrolysis of biological materials with acid or enzymes forms fluorescent products which interfere with the fluorometric determination of riboflavin. These compounds must be destroyed by oxidation or removed by selective adsorption of riboflavin from extracts, followed by elution of riboflavin from the adsorbant.

Development of the short method described subsequently in this report was based on two assumptions; the first of these was that riboflavin added to a food product would not be "bound" and could be removed readily by extraction with dilute acid. Secondly, it was assumed that if a relatively high level of riboflavin were added to a food product, the extract could be sufficiently diluted so that interference by naturally occurring compounds would not be appreciable and could readily be accounted for by a minor correction.

[1]Manuscript received April 19, 1962. Presented at the 47th annual meeting, St. Louis, Mo., May 1962. Contribution from General Mills, Inc., Central Research Laboratories, Minneapolis 13, Minn. Journal Series No. 287.

[2]Deceased.

Materials and Methods

A Pfaltz and Bauer Fluorophotometer was used. The same filters that are used with it for the A.O.A.C. method were used in the short method: No. 5113[3] in filter holder No. 6254; No. 3389[3] between lamp and cuvet housing; No. 3486[3] in cuvet holder.

The galvanometer of the Fluorophotometer was set at 0; then a cuvet containing a solution of 0.05 γ of sodium fluorescein per ml. of water was placed in the light beam and the amount of light passing through the lens was adjusted so that the galvanometer read 25 units.

Standard solutions of Merck U.S.P. riboflavin dissolved in a mixture of 1 part of 10% acetic acid to 2 parts of A.R. methanol were read with the galvanometer set at 25 as described before each reading. The values obtained were plotted against the concentration of riboflavin in the solution. The blank for the solvent alone was 2 units. The resulting straight line was used to establish the concentration of riboflavin in unknown solutions.

For other types of fluorophotometers a standard curve may be obtained in the same manner, but different concentrations of sodium fluorescein or riboflavin might be required in order to obtain deflections of the galvanometer of proper magnitude.

The cereal for which this method was developed contained a minimum added amount of 1.2 mg. of riboflavin per oz. incorporated in the cereal prior to flaking and toasting. A 2-g. sample of the toasted cereal flakes was mixed at low speed for 5 minutes in a Waring Blendor with 200 ml. of 10% acetic acid solution. The mixture was mixed for 10 minutes at a speed as high as possible without loss of sample by splashing. During extraction the mixture was protected from light.

An alternate method of extraction was also used in which 2 g. of finely ground cereal were shaken for 15 minutes on a mechanical shaker with 200 ml. of 10% acetic acid solution.

The mixture was allowed to settle for approximately 2 minutes, then a 25-ml. aliquot of the supernatant solution was withdrawn and mixed with 50 ml. of A.R. methanol. The methanol precipitated colloidal and dispersed materials, so that the mixture could be filtered rapidly through Whatman No. 1 paper to obtain a clear, colorless filtrate.

An aliquot of 15 ml. of the clear filtrate was placed in a cuvet and its fluorescence determined after the galvanometer had been set to

[3] These numbers are Corning designations. Numbers 5113 and 3389 are typical filters used in the primary light beam and No. 3486 is used in the fluorescent light beam.

read at 25 units with a solution of 0.05 γ of sodium fluorescein per ml. of water.

Samples of the cereal which had not been fortified with riboflavin were extracted and the extracts treated in the manner described. Control solutions consistently gave readings of 4 units, of which 2 units were presumably due to the solvent. The 2 units of deflection of the galvanometer due to the extracted control cereal was equivalent to approximately 0.07 mg. of riboflavin per oz. of cereal. Determination of the amount of riboflavin in the unfortified cereal by the A.O.A.C. method gave a value of 0.06 mg. per oz.

If one desires to determine only added riboflavin, the blank obtained on the control sample (2 units in this case) may be subtracted from the reading of each unknown solution.

The following calculations were used to convert γ of riboflavin per ml. of final extract of each sample to mg. of riboflavin per oz. of the cereal.

$$\gamma/\text{ml.} \times 1/1000 \times 3 \times 200 \text{ ml.} \times 28.4/2 = \text{mg./oz.}$$

The included graph, Fig. 1, is the standard curve used to calculate the results obtained at the General Mills, Inc., Research Laboratory. It is included merely to illustrate its preparation.

For simplicity in operation the galvanometer readings of the standard series of solutions of riboflavin can be plotted directly against mg. per oz., so the fluorescence of extracts of unknown samples can be read directly in mg. per oz.

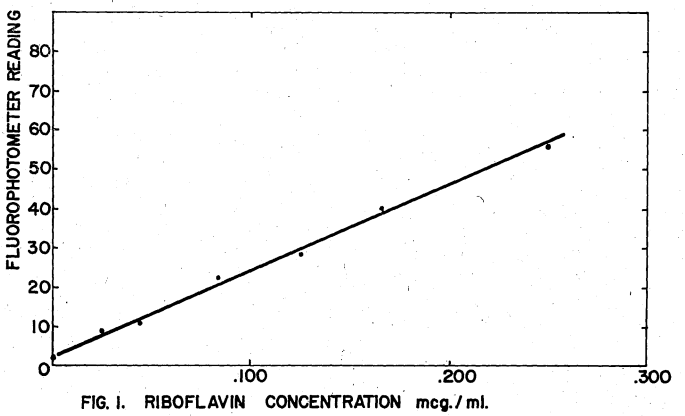

Fig. 1. Riboflavin concentration.

Results and Discussion

Tables I and II consist of a series of experimentally prepared and

TABLE I

ANALYSES OF RIBOFLAVIN IN CEREALS

SAMPLE DESCRIPTION	RESEARCH LAB. "QUICK METHOD"	THEORETICAL AMOUNT ADDED	A.O.A.C. METHOD, MERCK LAB.
	mg/oz	*mg/oz*	*mg/oz*
Pilot plant cereal			
1	1.14–1.12[a]	1.08	1.19
2	1.38–1.37[a]	1.28	1.37
3	1.72–1.72[a]	1.71	1.86
4	2.16–2.18[a]	2.16	2.18
Commercial plant cereal			
5	1.50	1.44	1.47
6	1.46	1.44	1.43
7	1.43	1.44	1.45
8	1.50	1.44	1.47
9	1.38	1.44	1.42
10	1.42	1.44	1.45

[a] These results were on extracts obtained by shaking.

TABLE II

ANALYSES OF RIBOFLAVIN IN CEREALS

SAMPLE DESCRIPTION	RESEARCH LAB. "QUICK METHOD"	THEORETICAL AMOUNT ADDED	A.O.A.C. METHOD, MERCK LAB.	PLANT QUALITY CONTROL LAB. "QUICK METHOD"
	mg/oz	*mg/oz*	*mg/oz*	*mg/oz*
Commercial plant cereal				
1	1.74	1.73	1.79	1.72
2	1.67	1.73	1.71	1.70
3	1.83	1.73	1.75	1.84
4	1.64	1.73	1.76	1.70
5	1.82	1.73	1.75	1.72
6	1.65	1.73	1.73	1.70
7	1.56	1.73	1.69	1.60
8	1.82	1.73	1.76	1.72

commercially prepared samples of toasted flaked cereal which were used for collaborative interlaboratory riboflavin analyses.

In the Central Research Laboratory 2-g. samples were selected at random from 8-oz. packages of samples 5 through 10, Table I, and the eight samples of Table II, to test the variability of distribution of riboflavin in the cereals. In sampling of samples 1 through 4, Table I, 8-oz. packages were ground and representative 2-g. samples used for the riboflavin determination.

In the Plant Quality Control Laboratory, 8-oz. packages of cereal were ground and representative 2-g. samples taken. Single determinations were made.

All the results listed under the heading of the Research Laboratory,

with the exception of the first four (see footnote, Table I), were obtained on extracts prepared in a Waring Blendor. All the results listed under the heading of the Plant Quality Control Laboratory were obtained on extracts prepared by shaking.

The method described in this report may be applicable to other food products which contain added riboflavin; but it has been applied to only one cereal product wherein the level of added riboflavin was approximately 25 times that of the level of naturally occurring riboflavin.

The loss of riboflavin due to light during normal manipulation in this method is insignificant in normal room lighting. However, it is desirable to use red or amber glassware if there is direct sunlight in the room or if solutions are allowed to stand between steps in the determination.

In extracting added riboflavin from a fortified cereal there is the possibility of direct extraction with an alcoholic solution rather than extraction with dilute acetic acid followed by clarification with alcohol.

Use of aqueous ethyl alcohol, 70 to 80%, has been recommended by a number of workers for extracting free amino acids from plant materials. Talley, Carter, and Porter (2) reviewed the reports on this procedure and showed that in the case of potatoes, multiple extractions were necessary in order to obtain complete extraction of all the amino acids.

Although cooked cereals do not contain active enzymes, it would be advantageous to use alcoholic extractions for isolation of components because of the difficulty of filtering water extracts. However, it was found in our laboratories that extraction of cereals with alcoholic solutions did not quantitatively remove free amino acids. A single water extraction resulted in quantitative removal of amino acids, but extracts obtained could not be readily clarified by filtration or centrifugation. Addition of ethanol until dispersed material precipitated (about 50% ethanol) resulted in easily filterable mixtures.

Because of past experience in extracting and isolating components of cereals, extraction with dilute acid followed by clarification with alcohol was used in the present method rather than direct extraction with alcoholic solutions.

Acknowledgments

Grateful acknowledgment is made to the following: Merck & Co., Inc., for collaborating in the analyses of samples; Miss Beate Feller of Merck Technical Service Laboratories, Rahway, N. J., for conducting the A.O.A.C. analyses; and the Quality Control Laboratories of General Mills, Inc., for helpful suggestions.

Literature Cited

1. Association of Official Agricultural Chemists. Official and tentative methods of analysis (9th ed.). The Association: Washington, D.C. (1960).
2. Talley, E. A., Carter, Fairie L., and Porter, W. L. Determination of end point in extraction of free amino acids from potatoes. J. Agr. Food Chem. **6:** 608–610 (1958).