A Note on Determining Protein Contents in Various Wheat Flours and Flour Streams by the Kjeldahl and by Neutron-Activation Methods[1]

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Aside from test-baking, protein analysis is the most reliable test for wheat quality. Each year thousands of wheat and flour samples are analyzed by the Kjeldahl method for protein contents to provide information for wheat breeding, grading, milling, baking, or feeding use.

The Kjeldahl method is precise, but time-consuming. It seems desirable to replace the Kjeldahl method with a short method for protein analysis. Furthermore, catalysts commonly used to facilitate digestion in the Kjeldahl method are mercury and selenium (or their compounds) or copper sulfate (1,2,3). Mercury (mercuric oxide) is probably most widely used for this purpose, but it is toxic. This evaluation of a new method as a possible alternative to the Kjeldahl method was stimulated by a desire to diminish the risk of environmental pollution, increase safety, and reduce the time involved for a complete analysis.

The neutron-activation method, deviating radically from the classic chemical procedure, has emerged recently to offer the possibility of a faster and simpler technique for protein analysis. Doty et al. (4,5) reported that a good correlation was demonstrated between protein contents in feedstuffs determined by the Kjeldahl and neutron-activation methods. However, there is no available information on applying the neutron-activation method to wheat-flour analysis. Thus, it seemed desirable to examine whether flour samples obtained from different wheat varieties, types, and various flour streams of different particle sizes and characteristics have any effect on the precision of the neutron-activation method, as compared with the Kjeldahl method.

MATERIALS AND METHODS

Flour Samples

Flour samples, as indicated in Table I, were milled from different varieties of hard red winter, hard red spring, soft red winter, white (club and common), and durum wheats.

Flour streams were collected from the milling of a blend of 84% hard red winter and 16% Gaines (white wheat) in Kansas State University's 200-cwt. pilot mill.

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Flour streams of distinctively different particle size and characteristics–such as 1st break, 5th break, 1st mid-top, 1st mid-bottom, bran and shorts duster, and straight flour–were selected for protein analysis. Their average particle sizes were measured by a Fisher Subsieve sizer (6) to be 13.5, 12.6, 17.5, 20.0, 10.0, and 16.5 μ for 1st break, 5th break, 1st mid-top, 1st mid-bottom, bran and shorts duster, and straight flour.

Kjeldahl Method

Crude protein was determined by the improved Kjeldahl method described in AACC Method 46-11 (2). The catalyst used for digestion was mercuric oxide.

Neutron-Activation Analysis

Two weighings of each sample were analyzed singly on two different days–for a total of four values–by the following procedure:

Kaman Nuclear's model 711 system (Kaman Science Company, Colorado Springs, Colo. 80907) was utilized for the activation analysis. This system is similar to that described by Doty et al. (4,5) and consists of a sample packing device, a pneumatic transfer system, a neutron generator, and a console containing loading and return ports, counting equipment, scalers, and the associated timing and control equipment.

A 17-g. sample was compressed into a specific volume in a polyethylene bottle so as to completely fill the bottle, with no associated air space. The bottle was injected into one of two loading ports. In the other loading port a melamine plastic reference, previously fitted into a similar polyethylene bottle, was also injected. This reference, together with the sample, was pneumatically conveyed to the neutron generator for irradiation.

The sample and the reference were irradiated for 50 sec. in a fast neutron beam (10^{11} neutrons per cm.^{2} per sec.) initiating the reaction $N^{14}(n,2n)N^{13}$. After the irradiation cycle was completed, both samples were pneumatically returned to the counting station near the console. There the samples were held for 12 min. to allow the short-lived interfering radionuclides to decay. N^{13} has a 10 min. half-life and decays by positron annihilation. These radiations are detected and measured by a NaI crystal-photomultiplier combination, which counts the radiations for 50 sec. after amplification and pulse-height selection.

Since the two most prevalent interferences – phosphorus and silicon – have short half-lives and decay rapidly during the 12-min. holding cycle, residual counts from these interferences are made on a separate scaler and corrected for in the calculations. A blank correction of the calculated protein value is also necessary in the calculation owing to interferences from the conversion of C^{13} to N^{13} by proton recoil.

The formula used in calculating the percent protein is as follows:

$$\% \text{ Protein} = [C_2 - K(C_3)/(C_1)\,(\text{Sample weight})] \times [CF - B]$$

C_1, C_2, C_3 are scaler counts where C_1 = count from the nitrogen in melamine reference; C_2 = count from the nitrogen in the sample; and C_3 = count from interference in the sample.

K is a factor relating the contribution of the interference count to the sample nitrogen count, and for this study was 0.7. CF is a calibration factor relating the corrected count to the protein content. B is a blank correction factor.

The above equation represents a straight line where CF and B are the slope and intercepts of the line, respectively.

RESULTS AND DISCUSSION

Table I shows that protein values obtained by the Kjeldahl and the neutron-activation methods agreed well, within experimental errors. The agreement indicated that neither flours milled from different varieties of three wheat types nor different flour streams of various particle size and characteristics had any effect on the neutron-activation method for nitrogen determinations.

When data obtained by the Kjeldahl method were plotted against those obtained by the neutron-activation method, all points lay very close to the theoretical line of

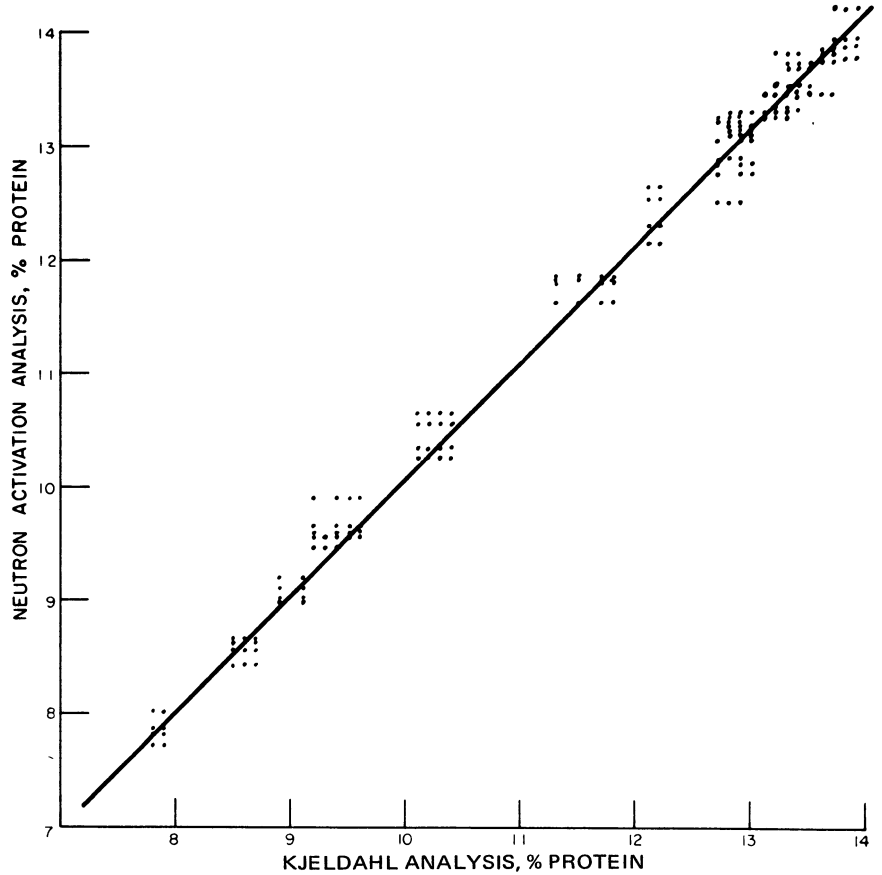

Fig. 1. Correlation of protein data obtained by the Kjeldahl and the neutron-activation methods.

TABLE I. TOTAL PROTEIN CONTENTS (N × 5.7) DETERMINED BY THE KJELDAHL METHOD AND THE NEUTRON-ACTIVATION ANALYSIS

Sample (Investigation) Number	Moisture %	Kjeldahl %	Neutron Activation %
Hard red winter			
Chiefkan × Tenmarq	12.8	13.1	13.3
(K.S. 501099)		13.2	13.3
		13.3	13.5
		13.2	13.1
Quivira-Tenmarq ×	12.7	12.7	12.9
Marquillo-Oro		12.7	12.9
(C.I. 12995)		13.0	12.8
		12.9	13.1
Chiefkan × Tenmarq	12.9	13.0	13.1
(K.S. 501097)		12.8	13.2
		12.9	13.2
		13.0	13.3
Comanche	13.4	12.7	12.9
(C.I. 11673)		12.9	13.3
		12.8	13.3
		12.8	12.5
Hard red spring			
Thatcher	12.0	13.4	13.8
(C.I. 10003)		13.4	13.6
		13.2	13.3
		13.3	13.5
Selkirk	12.1	13.6	13.8
(C.I. 13100)		13.6	13.8
		13.7	13.9
		13.6	13.5
Lee	11.8	13.3	13.6
(C.I. 12488)		13.4	13.5
		13.5	13.7
		13.5	13.7
Soft red winter			
Seneca	12.0	9.1	9.0
(C.I. 12529)		9.1	9.1
		8.9	9.0
		9.0	9.2
Durum			
Wells	15.1	11.8	11.8
(C.I. 13333)		11.5	11.9
		11.8	11.8
		11.7	11.7
White (club and common)			
Omar	9.6	8.5	8.7
(C.I. 13072)		8.6	8.4
		8.7	8.6
		8.7	8.7
Flour streams			
1st break	15.5	7.8	7.9
		7.8	7.8
		7.9	8.0
		7.8	7.7

TABLE I, Continued

5th break	13.9	13.8	14.2
		13.8	14.0
		13.7	13.9
		13.9	13.8
1st mid-bottom	14.2	9.2	9.7
		9.4	9.6
		9.5	9.6
		9.6	9.9
1st mid-top	14.4	9.2	9.6
		9.4	9.5
		9.3	9.5
		9.4	9.5
Bran and shorts duster	13.6	12.2	12.7
		12.1	12.6
		12.2	12.2
		12.2	12.3
Straight flour	14.3	10.2	10.6
		10.3	10.3
		10.3	10.7
		10.4	10.4

correlation of 1.0, as shown in Fig. 1. In fact, the correlation coefficient for protein values obtained by the Kjeldahl and neutron-activation methods was calculated to be 0.9961** (significant at 0.01 level).

The percentage coefficient of variation for the neutron-activation method was 1.48, and the variance components of the analysis could be broken down thus:

Variance components	*Variance*	*% of total*
Duplicates	0.029961	0.64
Different days	0.0	0.0
Different samples	4.421935	99.36
Total	4.449165	100.00%

The sample variance should be the largest on different samples; which, indeed, it is. As can be seen, the remainder of the various components was nil. This means that any variation obtained in the neutron-activation method could be attributable to sample difference and not to the method.

Because the neutron-activation method offers such advantages as rapidity, automation, simplicity, less pollution, and safety (4), as well as a preciseness equal to the Kjeldahl method demonstrated for determining protein contents in various wheat flours and flour streams in the present study, the neutron-activation method could be used advantageously to determine the protein content of a great number of wheat or flour samples for breeding, grading, baking, or feeding evaluation.

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