ANALYSIS OF PROTEIN CONTENT IN GRAIN BY PROTON ACTIVATION

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Abstract

The total protein in grain is an important measure of its nutritional value. In Canada, more than one million protein analyses are done annually, mostly by the traditional Kjeldahl chemical method. We have developed a new technique for protein (nitrogen) measurement, employing proton activation analysis. A beam of 16 MeV protons incident on the sample produces radioactive ^{14}O nuclei through the $^{14}N(p,n)^{14}O$ reaction. The ^{14}O decay ($t_{\frac{1}{2}}$=71 sec.) is detected off-line by its characteristic 2.31 MeV γ-ray. The proton flux is determined by the beam current collected in the sample. This gives an unambiguous measure of the abundance of nitrogen. The accuracy is comparable to the Kjeldahl method. Both whole grain and ground samples may be analysed. Weighing of samples is unnecessary since its effective thickness is determined by the proton range. A mechanized system for sample handling, necessary to handle the large number of samples, is under construction. The system, which will be controlled by the laboratory PDP15/40 computer is designed to process from 4 to 10 samples per minute.

Introduction

In order to measure the protein content of an organic sample, the total weight of nitrogen is determined, then multiplied by an accepted factor to give the protein content. Total nitrogen has traditionally been measured by the classical Kjeldahl chemical technique, a slow and involved process when applied to large numbers of samples. The measurement of the protein content of cereal grains is of increasing importance. In Canada more than a million such analyses are carried cut each year.

Various nuclear reactions have been proposed for use in protein analysis. In some cases the reaction products are observed directly, for example deuterons from the $^{14}N(p,d)^{13}N$ reaction.[1] More commonly the induced radioactivity is detected[2], for example annihilation radiation from the decay of ^{13}N. Since the annihilation radiation is not unique to ^{13}N, the ^{13}N activity may be confused with other activities produced.

We have investigated the use of the reaction $^{14}N(p,n)^{14}O$ for protein analysis [3]. [This is a reaction which has already been used for the detection of trace amounts of nitrogen[4),5)].] Here the amount of ^{14}N is measured by the intensity of the 2.31 MeV γ-ray emitted in the ^{14}O decay. The γ-ray provides in principle a unique identification of ^{14}O. At proton energies below 21 MeV, no other reaction can yield ^{14}O, so the intensity of 2.31 MeV γ-rays provides also an unambiguous measure of the amount of nitrogen in the sample. The 71 sec half-life is convenient for rapid analysis.

Method

A beam of 22 MeV protons was produced by the University of Manitoba cyclotron and analysed by a deflecting magnet. The beam, after being collimated, passed through an aluminum degrader which reduced the mean energy of the beam to 16 MeV, then through a thin window into air. The irradiation chamber, which held the sample and acted as a Faraday cup, was directly behind the window. The effective mass of grain irradiated was determined by the difference in range of protons at 16 MeV and protons at the reaction threshold ($\approx$6.4MeV), and amounts to $\approx$0.23 gm/cm^3. This eliminates the need to weigh the sample. The proton beam hitting the sample ($\sim$0.1μA) was determined by measuring the charge collected in the Faraday cup.

The ^{14}O nuclei produced in the $^{14}N(p,n)$ reaction decay (99.4%) to a level at 2.31 MeV in ^{14}N. The de-excitation γ-ray from this level was detected off-line. The ratio of 2.31 MeV γ-ray intensity to the total beam collected in the Faraday cup determined the percentage of nitrogen in the sample.

Results

Samples of cereal grains, rapeseed, and a high protein flour were analyzed, both with the present method and the Kjeldahl method. Repeated measurements on the same sample with the present method give reproducibility consistent with the counting statistics ($\sim$1%). No difference was found between samples of whole and ground grain. A comparison of the measurements by the present method and the Kjeldahl method is shown in Figure 1. The straight line least squares fit has a coefficient of variability of 2.4%.

Corrections were made to the data for pileup, the dependence of proton range on material and the presence of nitrogen in the air between kernels. The only noticeable background activity came from ^{38}K, which has a γ-ray of 2.17 MeV and a half-life $\sim$460 sec. It may be formed in the $^{39}K(p,d)^{38}K$ reaction or the ^{40}Ca (p, He) reaction, which have thresholds of $\approx$ 11MeV and $\approx$ 14 MeV. The "tail" of the γ-ray spectrum was found to give a measureable contribution to the ^{14}O γ-ray spectrum at

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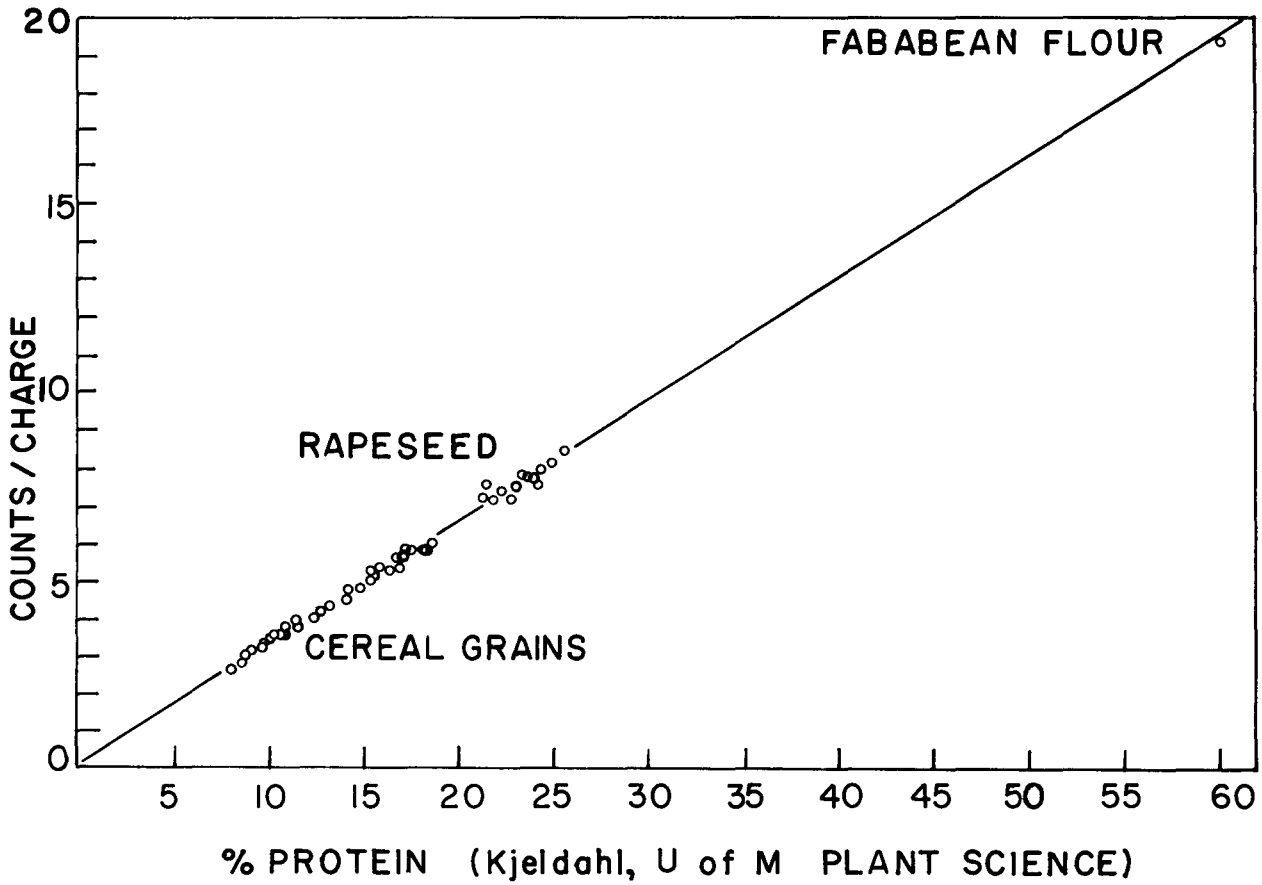

Figure 1. Comparison of measurements with the present method (counts/charge) and the Kjeldahl method.

22 MeV, but at 16 MeV the contribution was small enough to be neglected.

Automation

The above results were obtained manually placing the samples in the irradiation chamber and moving them to the counter position. Since the sample irradiation takes place in air and since it is not necessary to weigh the sample, it is quite straightforward to automate the analysis. An apparatus to mechanize the process has been constructed and is now being tested. The sample material is placed in a plastic cup, 4.2 cm in diameter by 1.3 cm deep. The sample is held within the cup by means of an adhesive paper lid which is attached to the top of the cup. The cup is placed within a metal ring forming part of the Faraday cup. Samples are transported to and from the irradiation area by means of the conveyor belt. The entire operation of beam exposure, sample positioning and transport, counting and data analysis and storage are under control of the laboratory PDP 15/40 computer. Four NaI counters (12.7 cm diameter by 7.6 cm long) have been installed. In initial tests with the apparatus, a single protein measurement has been made in 30 seconds. The system is designed so that the sample will be irradiated while the activity of the previous sample is being counted, so that an initial analysis rate of 4 samples per minute is expected.

References

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DISCUSSION

B. LARSSON: I am a member of a group at Uppsala who has studied this important area of application for some years. We have the general view that several charged particle-induced prompt reactions (as d,p or p,d) may be used to an advantage for studies on single grains but that, for bulk specimens, the well-known $^{12}N(n,2n)^{13}N$ or $^{14}N(\gamma,n)^{13}N$ reactions offer the best prospects, taking also the economical and practical factors into account. Would you be so kind as to comment on this?

D.A. DOHAN: One of the requirements in doing protein analyses of grain samples, is precision. Our data indicate that the (p,n) activation analysis method is capable of producing results with an accuracy comparable to the Kjeldahl results, which is of the order of 1-2% of the amount of nitrogen. The results for the (n,2n) and (γ,n) experiments had errors which were significantly larger than these, and my feeling is that it would be difficult to obtain sufficient accuracy with these methods.

Concerning single grain analysis, we have done experiments in which we irradiated single kernels for which the embryo portion of the grain was shielded from the beam. Results of growth tests indicate that it is possible to irradiate half-shielded kernels with doses up to 6 Mrad without affecting growth of the plant. Therefore, in theory, one could measure the protein of a single kernel, and then grow it in a plant-breeding or genetics experiment.