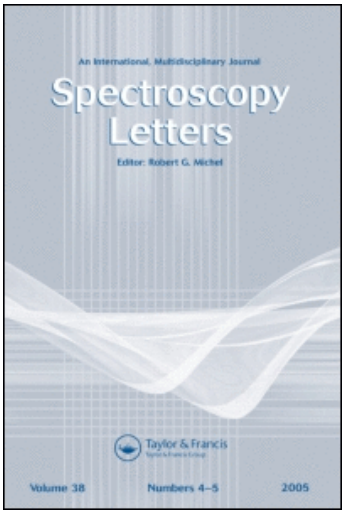

Spectroscopy Letters
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597299

Determination of Protein Levels in Nigerian Grains Using 14 MeV Neutron Activation Analysis

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To cite this Article Ewa, I. O. B.(1996) 'Determination of Protein Levels in Nigerian Grains Using 14 MeV Neutron Activation Analysis', Spectroscopy Letters, 29: 5, 885 — 900
To link to this Article: DOI: 10.1080/00387019608001619
URL: http://dx.doi.org/10.1080/00387019608001619

SPECTROSCOPY LETTERS, 29(5), 885-900 (1996)

DETERMINATION OF PROTEIN LEVELS IN NIGERIAN GRAINS USING 14 MeV NEUTRON ACTIVATION ANALYSIS

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KEY WORDS: Fast neutrons, Positrons, Nitrogen, Kjeldahl, Nigerian grains, Soya beans, Sorghum, Maize, Beans.

ABSTRACT

Fast (14 MeV) neutrons have been successfully used in investigating the protein content of different food grains (Soya Beans, Sorghum, Maize and Beans) locally grown and consumed in Nigeria. Protein was estimated via nitrogen using the $^{14}N(n,2n)^{13}N$ reaction. Quantification of nitrogen was achieved through a γ-γ coincidence counting of 511 keV positron emissions from the decaying ^{13}N. The implication of the use of the emitted annihilation positrons, the interference introduced in the analytical energy spectra from other activated target nuclides present in the sample, as well as possible proton "knock on" reactions anticipated from cellulose in grain matrix were assessed, and their contributions to the 511 keV gamma energy resolved. For comparative purposes, replicates of samples analyzed through

Fast Neutron Activation Analysis (FNAA) were investigated using the Kjeldahl method. The samples were carried through the Kjeldahl process of pre-digestion (with appropriate catalysts), digestion and titration. The results obtained through the Kjeldahl process were found to be in good agreement with those obtained using FNAA although slightly lower. Protein content(%) of Nigerian grains analyzed varied from a low 8.75 ± 2.96 for sorghum to 35.93 ± 0.31 for soya beans.

INTRODUCTION

The determination of protein content in food-grains will continue to attract agricultural service analyst of developing countries like Nigeria, due to their population's dependence on seeds as one of the principal sources of nutritional protein. To this end, the need will arise for the evolution and establishment of fast, accurate and reliable screening methods for the determination of protein levels in competing grains and cereals grown.

One of the earliest, classical and most widely used method for protein analysis equally developed in Nigeria is the Kjeldahl method. Though accurate, the limitations of this method include possible errors associated with the "wet chemistry" approach, automation restrictions and the relatively low sample rate analysis per day. Niemann [1] had earlier reviewed the potentiality of the use of Neutron Activation Analysis (NAA) as well as other techniques such as Particle Induced X-ray Emission (PIXE), Photoelectron

Spectroscopy (ESCA), Nuclear Magnetic Resonance (NMR), Nuclear Quadrupole Resonance (NQR) and Mossbauear Spectroscopy as being equally useful and competing in the probing of protein content of plant materials in one form or the other.

If an option should favour the use of NAA for protein analysis of Nigerian grains, then the limitation of its application vis-a-vis the classical Kjeldahl method must not only be evaluated in terms of cost but also in terms of the long-term investment for the method developed and high-rate sample analysis per day. It is well known that the most essential requirement for the use of fast neutrons in protein analysis is a 14 MeV neutron generator. With a neutron flux in the range of 10^8 n $cm^{-2}s^{-1}$, it becomes useful in the investigation of protein in grains via the nitrogen obtained from the endothermic, particle emission $^{14}N(n,2n)^{13}N$ reaction. Having a threshold energy of about 10.6 MeV and a cross-section of about 5.7 mb at 14 MeV neutron energy [2], this reaction results in a β^+decay of the ^{13}N radionuclide. Though very promising, the determination of nitrogen in grains through the detection of the 511 keV gammas from the emitted annihilation positrons during the decay of ^{13}N must be evaluated with caution, since different activated target nuclides in the sample matrix (grains) could lead to "parasitic emissions" through either higher yields of 511 keV gammas or interferences from identical ^{13}N daughter radionuclides resulting from other nuclides [3]. This was given due consideration during the protein analysis of the selected grains grown in Nigeria as reported in this work.

This paper therefore reports the use of two analytical methods: a nuclear(FNAA) and non-nuclear (Kjeldahl) method, for the analysis of replicate samples of grains for their protein content.

EXPERIMENTAL

Samples and Standards

Selected grains from Nigeria (different varieties) commonly grown and consumed for nutritional protein were used in this study. They are soya beans, guinea corn (*Sorghum bicolor*), maize (*Zea mays*) and black-eyed beans(*Phaseolus spp*). The guinea-corn, maize and bean samples were hand-pulverized as received in a porcelain mortar into fine particles. Grinding of the soya beans was performed mechanically using teflon crushing balls (0.95 cm diameter) in 30 ml flat-bottom teflon containers for 10 minutes. Thereafter, the samples were divided into two sets with each replicate subjected to both FNAA and Kjeldahl analysis.

(a) 14 MeV Neutron Activation Analysis(FNAA).

The prepared samples were compacted into clean pre-weighed polyethylene vials (3.2 cm x 1.4 cm). Nicotinic Acid (Batch No.15360) from Eastman Kodak Co. Rochester, N.Y; and Urea (NIST-912) were used as standards. Since the nitrogen concentration of the standards were relatively high 11-37%N for Nicotinic Acid and 46.642%N for Urea, Nitrogen dilution in the standard matrix was achieved using Methyl cellulose (Batch No. MX850 of MC/B) in order to arrive at

Nitrogen ranges of 1% through 11% for the standards. Methyl cellulose was used for matrix corrections arising from $^{12}C(p, \gamma)^{13}N$ and $^{13}C(p,n)^{13}N$ "knock-on" reactions as proposed by James and co-workers [4]. This correction is necessary since cellulose basically forms the matrix of plant materials such as grains and could therefore give misleading yields of ^{13}N from the reactions stated above. The standards were packed into identical sample volume in order to ensure consistent neutron irradiation geometry for samples and standards. Sample and standard vials were encapsulated into 2 dram irradiating rabbits.

Irradiation and Gamma Spectrometry

Standards and samples were irradiated with a neutron flux of about 10^8 n $cm^{-2}s^{-1}$ from a 14 MeV neutron generator (Model A-710, Kaman Sciences Corporation, Colorado Springs, USA) of Texas A & M University, Texas, USA. A stable beam current for the $^3H(d,n)^4He$ (neutron-emitting) reaction in the sealed tube was achieved at 0.22 mA(dc) with an ion accelerated voltage of 135 kV. Integrated fast neutron monitoring during the five minute irradiation periods was performed for each analysis by the use of a BF_3 proportional counter.

The main objective of the neutron monitoring for each irradiation was to validate the fluctuation in the neutron output for each sample run and thereby providing data for the neutron flux normalization factor for all the runs. Delay and counting schemes were optimized in such a manner as to eliminate the interfering $^{31}P(n,2n)^{30}P$ reaction.

A gamma coincident unit consisting of a two 7.6 cm by 7.6 cm NaI(Tl) detector (fig. 1) was used in counting the positrons with a window setting for 511 keV gamma rays. The positron annihilation counts were acquired using an ND66 Multichannel Analyzer (Nuclear Data Inc., Schaumburg, Illinois), with preset energy channels corresponding to the irradiation, delay and counting time schemes using a 6 channel per minute time lapse for each run.

Theory of Method and Calculations

The basic gamma radiation used in the investigation of protein in grains comes from the positron emitting $^{14}N(n,2n)^{13}N$ reaction. Being radioactively unstable, ^{13}N undergoes an isobaric transformation to the stable ^{13}C through the usual β^+decay as given below (the neutrino {υ}, inserted in the equation as expected by the conservation laws):

$$^1p \rightarrow {}^1n + e^+ + \upsilon$$

The emitted positrons (e^+) interact with negative eletrons (e^-); both particles having their rest mass (M_o) converted to electromagnetic radiation equivalent to $2M_oC^2$, where c represents the speed of light in vacuum. Two gamma quanta results, each having an energy of 511 keV and annihilated at 180° to each other. This is the basis for which the gamma coincident counting geometry of two detectors at 180° is usually adopted for the quantitation of the annihilation positrons. The major problem associated with the use of 511 keV positron detection *per se* in the determination of total nitrogen via the $^{14}N(n,2n)^{13}N$ reaction stems from the

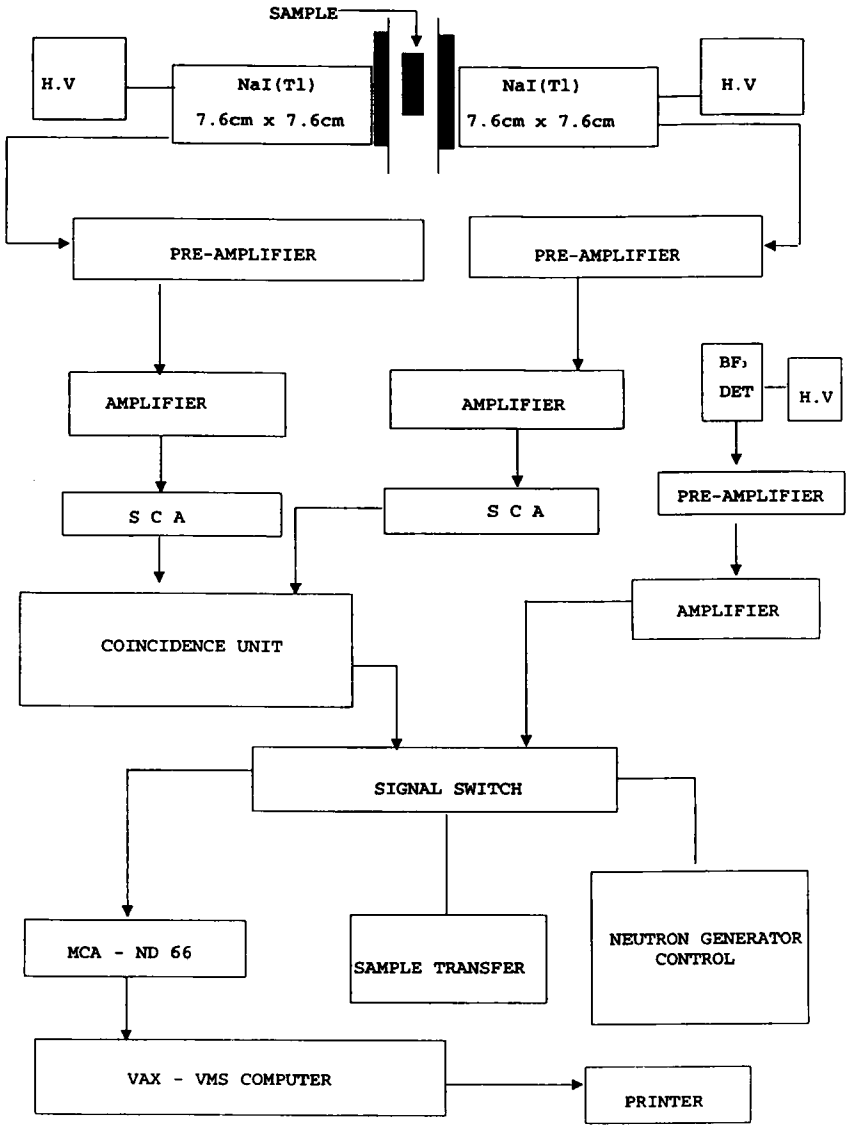

Fig. 1 Block diagram for the coincident counting and flux monitoring

fact that as many as fifty three theoretically predicted reactions [2] could be associated with the emission of 511 keV gamma rays.

While the possibility of these reactions occurring and consequently interfering with the $^{14}N(n,2n)^{13}N$ reaction could be screened by the use of pertinent nuclear parameters like the reaction cross-section(table 1) for the 14 MeV neutrons, half-life,and isotopic abundances of such target nuclides in the grains, two reactions emanating from the presence of potassium and phosphorus(ref. table 1), remained persisting, and unresolved. The phosphorus and potassium positron emitting reactions interfere with the 511 keV gamma ray inventory derived from the $^{14}N(n,2n)^{13}N$ reaction. Elimination of these interferences was achieved by:

i) choosing an appropriate decay interval (equivalent to about five half-lives of the decaying ^{30}P) for the elimination of 511 keV positrons arising from its β^+ decay

ii) using a peak area correction procedure to obtain a potassium peak area normalization ratio for evaluating potassium-free sample counts. This was achieved by separately irradiating a potassium standard of known concentration identical to that present in the grains and using the gamma energy of ^{38}K at 2167 keV to normalize the potassium contributions for the 511 keV positrons counted during the nitrogen analysis of the grains.

A correction of possible "knock on" reactions like $^{13}C(p,n)^{13}N$ and $^{12}C(p,\gamma)^{13}N$ due to matrix effects from grain cellulose was performed using methyl cellulose irradiated under the same experimental conditions and geometry.

Table 1.
Nuclear data of some reactions associated with the emission of 511 keV positrons during the nitrogen analysis

Element	Nuclear Reaction	Half-Life (mins)	Cross-section (mb)	Gamma Energy (keV)
Nitrogen	$^{14}N(n,2n)^{13}N$	9.96	5.7	511
Phosphorus	$^{31}P(n,2n)^{30}P$	2.50	11	511
Potassium	$^{39}K(n,2n)^{38}K$	7.71	4	511, 2167

Calculations

All the counts detected were normalized with BF_3 flux normalization constants for each irradiation. A mean standard activity (interference-free counts per gram nitrogen) evaluated for each of the activated standards was found to be 6.52×10^4 counts/g.Nitrogen, with an error limit of ± 5.5% . The specific activity for each of the samples was calculated using the relation:

Specific activity of sample $= \{A_x - [k(A_k) + A_c]\}$ counts/M

where

A_x = Flux normalized 511 keV counts of sample

k = Potassium peak area normalization ratio for cellulose/matrix free interference, determined experimentally to be 2.64

A_k = Flux normalized potassium peak-free counts for sample

A_c = Flux normalized cellulose peak-free counts for sample

M = Mass of sample.

Finally the nitrogen content in the grains was obtained by the relation:

$$\text{Nitrogen}(\%) = \frac{\text{Specific activity of sample}}{\text{Mean Activity of standard}} \times 100\%$$

The Mean activity of the standard was evaluated to be 6.52×10^4 counts/g Nitrogen. Nitrogen (%) derived from the above expression was then converted to protein (%) using the relation [N% x 6.25 = Protein%].

(b) Kjeldahl Analysis

Replicate samples of the pulverised grains (beans, sorghum maize and soya beans) from the same lot prepared for FNAA were used for the Kjeldahl analysis in order to allow for an unbiased comparison of the results.

The samples were oven dried for 2 hours at 130°C in order to reduce the moisture content of the seeds. They were then cooled in a desiccator for 1 hour and further crushed and ground to finer particles in a porcelain mortar. Lysine was used as standard for the Kjeldahl method. Taking into consideration the estimated nitrogen guarantees for the samples and standards, appropriate weight equivalents of both samples and standards were introduced into separate Kjeldahl flasks and were carried through the three principal stages of digestion, distillation and titration.

The digestion process began with the introduction of a mixed protein catalyst(K_2SO_4 / $CuSO_4$) into each Kjeldahl flasks containing the samples and standards. Boiling stones and

conc. H_2SO_4 were added and the solution made to boil in a pre-heated burner for 2.5 hours with occasional gentle rotation of the Kjeldahl flasks as the boiling proceeded. With the boiling process terminated, the flasks were cooled, followed by the addition of about 400 mL of water in order to convert all the nitrogen present in the analyte as amino acid protein into the ammonium ion through the form:

$$N(\text{protein}) \rightarrow NH_4^+$$

The next step was the distillation process. This began with the conversion of the ammonium ion (NH_4^+) in whichever complex that it existed into ammonium gas which was later collected as represented in the following ionic equations:

(a) Distillation : $NH_4^+ + {}^-OH \rightarrow NH_3(gas) + H_2O$

(b) Collection : $NH_3 + H^+ \rightarrow NH_4^+$

This stage was accomplished practically by pouring about 50% NaOH into the digested analyte fractions. About 25 mL of 0.4N H_2SO_4 and 75 mL water were added to the digested samples followed by the addition of 2-3 drops of methyl orange(indicator) and the distillation made to proceed at a 7.5 minute boiling duration.

The final stage in the Kjeldahl process was the titration. An automated Brinkman titrator was used, beginning with a starting point pH of 2.8 for methyl orange used as indicator to an end-point pH of 9.0 as the solution turns from red to yellow. A computer assisted program gave the calculated values of the concentration(%) of nitrogen in the samples from the titration results.

RESULTS AND DISCUSSION.

Protein values of the various grains determined using 14 MeV FNAA and the Kjeldahl method is reported in table 2. Values obtained by FNAA appear slightly higher than the ones from the Kjeldahl process. The differences could be explained as follows. Cereal proteins may either be classified on the basis of their morphology as endosperm proteins, proteins of the aleurone layer, or protein of the embryo. Another basis for classification [5]could be the chemical form in which they exist such as: simple protein, complex protein, lipoproteins, glycoproteins, nucleo proteins etc. Protein determinations in grains, using non-nuclear techniques involve a series of steps in extraction, separation, digestion, dissolution of significant fractions of the proteins depending on their solubility and chemical forms in which they exist. One of the problems associated with these methods is the inability of some of the experimental techniques to achieve total extraction. This could be the limitations associated with using the Kjeldahl process. Laundry and co-worker [6] used the Osborn method of extraction with little success, reporting a residual fraction of 5% of the total nitrogen unextracted, while investigating maize protein. However, total protein was achieved through nuclear techniques as reported in this study, since the method is independent of the chemical state of the proteins in their respective matrices.

Protein values for legumes (soya beans and black-eyed beans) are much more higher than values obtained, for the cereals (maize and sorghum). The mean value of protein in

Table 2.
Protein estimates for the Nigerian grains investigated using FNAA and KJELDAHL methods

Grain variety (n=4)	Nitrogen(%)	Protein(%)	
	(FNAA)	(FNAA)	(KJELDAHL)
Soya beans(light brown)	5.7 ± 0.05	35.94 ± 0.31	34.6
Soya beans(light green)	5.8 ± 0 04	36.25 ± 0.20	35.8
Maize (Local breed)	1.4 ± 0.03	8.75 ± 0.22	9.5
Maize(Hybrid)	1.8 ± 0.05	11.25 ± 0.20	10.6
Black-eyed beans(white)	4.9 ± 0.65	30.63 ± 0.50	30.3
Black-eyed beans(brown)	3.6 ±.0.70	22.50 ± 0.40	22.6
Sorghum(Guinea corn)	1.4 ± 0.21	8.75 ± 0.35	8.8

soya beans obtained by FNAA as obtained in this work is within the range of 34.9 to 46.5% as reported by Szegedi and co-workers [7], who used the same conversion factor of 6.25 for obtaining protein from nitrogen content. The protein values for maize obtained (8.75 - 11.25%) compares favourably to other protein levels determined for Brazillian maize ranging from 8.94 to 12.50% [8,7] and Hungarian maize (10.6% to 12.3%) as reported by Hecksch-Hadju [9]. It should be noted that the local variety of maize grown in Nigeria as reported, fell lower in its protein yield when compared to the hybrid species although it has been known that the latter has low starch content.

Protein levels in grains grown in different regions are actually not constant but vary within a limiting range.

Variations are usually associated with soil nitrogen, climatic conditions of the growing region, variety grown, and agrotechnical condition of planting including fertilizer application. A survey of world collection of sorghums [10,11] showed greater variability of 4.7 to 17.0% . A mean value of 8.75% was determined for sorghum in this study.

Cereals and Legumes will continue to be of research interest, contributing to about 70 to 90% of the total protein consumed in developing countries [5]. Such a high rate of dependence on grains will necessitate the use of fast screening techniques like the NAA in assessing varieties with high nutritional quality in terms of their protein levels.

ACKNOWLEDGEMENT

This project was sponsored by the Centre for Energy Research and Training, Ahmadu Bello University, Zaria, Nigeria and partly by the International Atomic Energy Agency, Vienna, Austria through a Research Fellowship (NIR/8831P) on nuclear analytical techniques. I wish to acknowledge the use of facilities of the Centre for Chemical Characterization, Texas A & M University, Texas for this work.

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RECEIVED: January 4, 1996
ACCEPTED: February 16, 1996