DETERMINATION OF PROTEIN CONTENT IN SEEDS BY PROMPT GAMMA-RAY SPECTROMETRY

Artur W.Carbonari and Brigitte R.S.Pecequilo

Nuclear Physics Division
Instituto de Pesquisas Energéticas e Nucleares
Comissão Nacional de Energia Nuclear
Caixa Postal 11049 - Pinheiros
01000 São Paulo - SP - Brasil

ABSTRACT

The protein level in seeds can be directly calculated through the determination of the nitrogen content in grains. We show here that the radioactive thermal neutron capture prompt gamma-rays technique can be used to determine the nitrogen content in grains without chemical destruction, with good precision and relative rapidity, by detecting the prompt gamma rays emitted by the $^{14}N(n,\gamma)^{15}N$ reaction product. The samples were irradiated in the tangencial tube of the IEA-R1 research reactor, in São Paulo, and a pair spectrometer was used for the detection of the prompt gamma-rays. The nitrogen content was determined in several samples of soybean, common bean, peas and rice and the results compared with typical nitrogen content values for each grain.

INTRODUCTION

1. Main Principles

The main nutricional deficiency affecting populations in developing countries centres on protein-calorie shortage. The shortage of calories results in undernourishment, while a diet deficient in proteins leads to malnutrition, which constitutes the general problem of protein-calorie deficit, since the human body, faces a calorie shortage, it will convert protein into calorie, but not the reverse[1].

The increased production of protein from plant source is the foremost solution for the protein-calorie malnutrition problem with an economic advantage: to produce 1 kg of animal protein, it is estimated that about 5 kg of plant

protein and eight to ten times as much water are required[2].

The protein content is directly relationed with the nitrogen content in seeds and a constant factor is usually used for this conversion within one plant species. Percent nitrogen, multiplied by a constant factor of 6.25 is an accepted and stablished method of expressing the protein content in the majority of seeds[3].

One of the most promising ways to reduce the protein deficiency in developing countries are the plant breeding programs. These programs need a constant monitoring of the protein content on large populations of seeds (sizes of the order of 10,000 in the second generation) without chemical preparation or destruction of the samples[4,5] to a suitable selection.

The first step in a plant breeding program is a nitrogen content determination, as a way of selection.To improve the hereditary nature of a breeding population, the low protein varieties should be eliminated by measuring the seed protein content of successives progenies[3,4,6,7].

As an alternative to the classical conventional chemical methods which spend a lot of time for sample preparation and chemical treatment, nuclear techniques have been used successfully. The main nuclear techniques used for the nitrogen determination in seeds are:

- fast neutron activation analysis[4,8,9,10]
- photonuclear activation analysis[4,11,12]
- proton activation analysis[4,13,14]
- thermal neutron capture prompt gamma-ray analysis[4,15,16,17]

The three early techniques are activation techniques which require a time after the irradiation to made the measurement, while in the latter the measurement is made during the irradiation.

In this paper we present a technique developed for determining the nitrogen content in several seeds by the thermal neutron capture prompt gamma-ray analysis.

In a thermal neutron radioactive capture reaction, or (n,γ) reaction, a thermal neutron is captured by a target nucleus and the compound nucleus decays to the ground state by emitting several gamma-rays with energies varying from a few keV to several MeV. Since these gamma rays are emitted within a very short time interval ($\sim 10^{-14}$s), they are so-called prompt gamma-rays and their detection is made simultaneously with the sample irradiation. The gamma-rays appear like peaks in a gamma-rays spectrum, with energies varying from low to high values. Since each isotope presents several characteristic gamma-rays, the energy determination of the peaks allows the element identification and the peak area is proportional to the quantity of the element considered.

The ^{14}N isotope is about 99.6% of natural nitrogen and captures a thermal neutron through the $^{14}N(n,\gamma)$ reaction. The ^{15}N reaction product formed is stable so,not detected by conventional thermal neutron activation analysis, but easily detected by prompt neutron capture gamma-ray analysis once the gamma-rays spectra are accumulated at time of irradi

ation.

The detection sensitivity of a single isotope depends upon its thermal neutron cross section (σ) and upon the sample nature, since inter-element interference can disturb the analysis of the single one.

Although the low ^{14}N capture cross section[18], the main advantage of using the prompt gamma-ray analysis technique for nitrogen determination in seeds is the 10.83 MeV ^{15}N transition[19], not existing for other elements.

Some other important advantages of that method are:

a) it is a direct method of analysis, in the sense that it is not necessary a previous chemical sample preparation,
b) it can be totally automatized,
c) it permits simultaneous analysis of other elements possibly present in the sample,
d) it permits the employ of an isotropic neutron source, instead of a reactor, with a very low cost and feasible to transport elsewhere,
e) the low absorption cross sections of the basic vegetables components (carbon, hydrogen and oxygen), the high energies of the emitted prompt gamma-rays and the large penetration power of the neutrons allow the measurement of large samples.

2. Nitrogen Mass Calculation

Assuming a certain target with N nuclei per cm^2, the gamma-rays emitted by unit time, for a radioactive capture reaction are equal to

$$\frac{dN}{dt} = N \sigma \phi \quad I_\gamma f \qquad (1)$$

where σ is the thermal neutron capture cross section, ϕ is the neutron flux, I_γ is the absolute intensity of the considered gamma transition and f is the isotopic abundance of the considered isotope.

However, the gamma-rays reaching the detector and able to be registered by the detection system are related also with other factors, so we have

$$\frac{dN}{dt} = N\sigma\phi \; I_\gamma f \; \Omega \; T_\gamma \varepsilon_\gamma \qquad (2)$$

Ω is the solid angle between the target and the detector, T_γ is a transmission factor envolving the attenuation of the gamma-ray beam through several absorvers until reaching the detector and ε_γ is the detection system efficiency.

Otherwise, the areas of all the peaks in the gamma-ray spectrum are proportional to the gamma rays emitted by the target, so, after a counting time t, we have

$$AREA = N\sigma\phi \; I_\gamma f \; \Omega \; T_\gamma \varepsilon_\gamma \; t \qquad (3)$$

But $N = \frac{m \; No}{M}$, with m being the mass of a certain isotope present in the sample, No the Avogadro's number and M the atomic mass of the element and we can rewrite

$$AREA = \frac{m \; No}{M} \sigma\phi \; f \; I_\gamma \; \Omega \; I_\gamma \varepsilon_\gamma \; t \qquad (4)$$

Then, by calculating the peak area we are able to determine the mass of the isotope of interest present in the sample.

EXPERIMENTAL

1. Samples and Standard

For the development of the method we used as neutron source, the IEA-R1 research reactor at São Paulo.

The experimental set up for this experiment is installed at the tangencial tube of the reactor and the basic features of our internal target facility were described in more details elsewhere, by Pecequilo[20,21].

For data acquisition, a pair spectrometer[20] and a 8192-channels analyzer were used.

The samples to be analyzed are positioned close to the reactor core and the neutron flux is typically about $5x10^{11}$ $n.cm^{-2}.s^{-1}$ at the target position.

The sample holder is of pure graphite with a 4.0 cm inner diameter and a depth varying from 0.3 to a maximum of 1.3 cm. The holder has also a small cavity for the nickel standard pellet used in our measurements.

This standard, having a known mass and being irradiated together the seeds sample, allows us to eliminate both the inaccurate value of the neutron flux and the time of the measurement (eq.4) through the nitrogen and standard areas ratio.

We have selected a nickel standard since it attends the following requirements:

- it is an element absent in the vegetal, so, both the nitrogen and the nickel transitions are free of mutuam interferences,
- it has a reasonable thermal neutron capture cross section for the most abundant isotope[22],
- the 9 MeV gamma transition of the ^{59}Ni compound nucleus has a high intensity; also, none of the other elements present in the sample emitts such a gamma-ray energy,
- the 9 MeV ^{59}Ni transition stays in the same energy region as the 10.83 MeV ^{15}N transition used in our calculations, so we can measure both of them at the same time.

Fig.1 presents a typical soybean spectrum, with both the 9 MeV and 10.83 MeV transitions, clearly without interferences.

The nitrogen and nickel peaks areas ratio is given by

$$\frac{A(N)}{A(Ni)} = \frac{m(N)\ M(Ni)\ \sigma(N)\ I_\gamma(N)\ f(N)\ \varepsilon'_\gamma(N)}{m(Ni)\ M(N)\ \sigma(Ni)\ I_\gamma(Ni)\ f(Ni)\ \varepsilon'_\gamma(Ni)} \qquad (5)$$

where A(N) is the nitrogen peak area and A(Ni) is the nickel one. The other symbols are the same at in equations 1-4.

The ε'_γ efficiency is a relative efficiency, because we are not consider the gamma-ray transmission corrections[23] and the nuclear data used in our calculation are from references 19 (nitrogen transitions intensities), 18 (nitrogen thermal capture cross section), 22 (nickel transitions inten

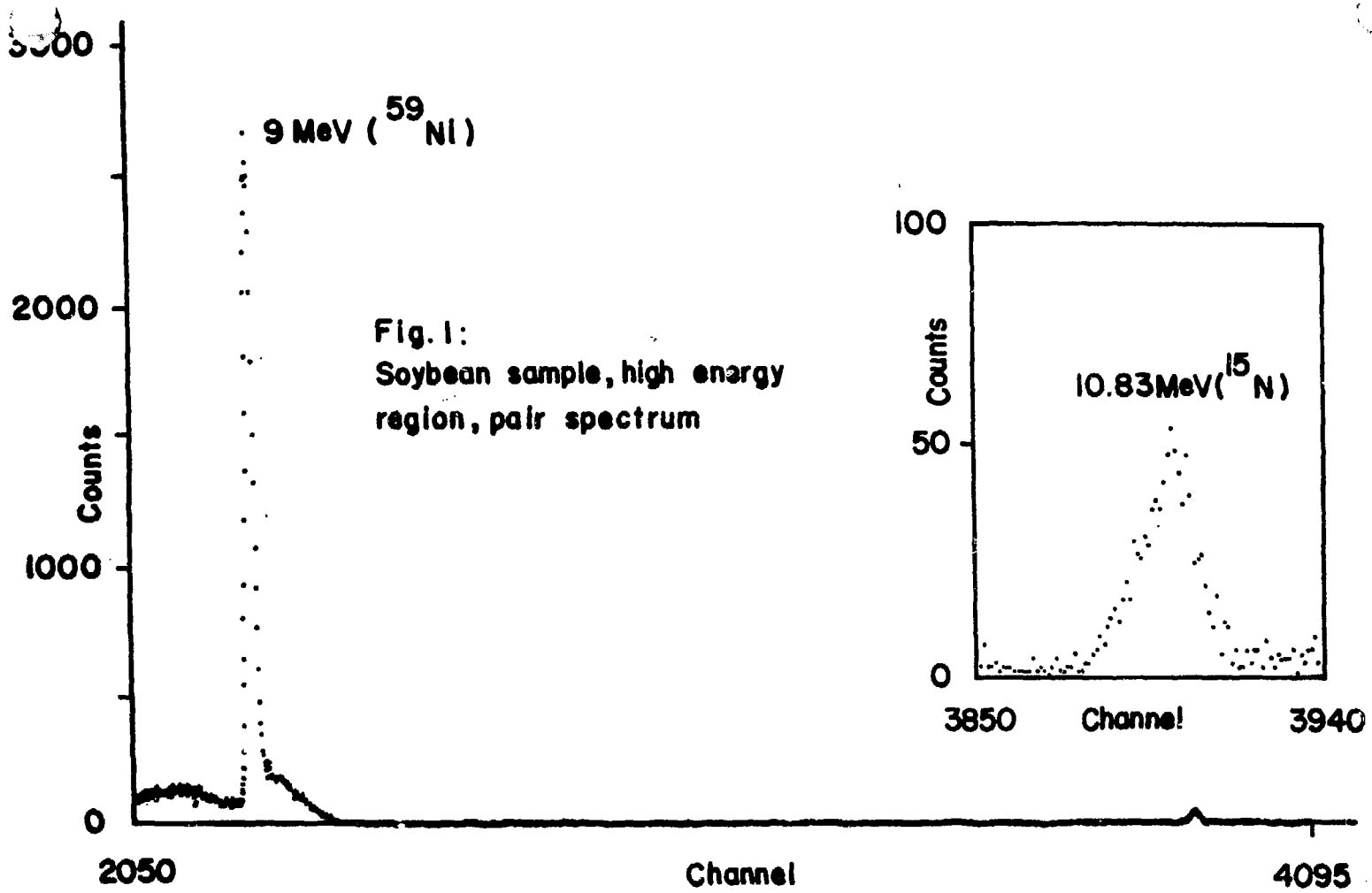

Fig. 1:
Soybean sample, high energy region, pair spectrum

sities,isotopic abundances, and capture cross sections) and 24 (nitrogen isotopic abundance).

2. The Pair Spectrometer Efficiency

The determination of the efficiency curve of our pair spectrometer is made through the measurement of a prompt gamma-ray energies set with well-known energies and intensities.

We used a compound target of melamine($C_3H_6N_6$), sodium chlorine (NaCl) and metallic chromium measured in the high energy region (6.5 to 11 MeV), for in this region, the nitrogen, chlorine and chromium isotopes have a large number of transitions with very well determined intensities and energies.

The relative efficiency ε'_γ of a certain energy with an I_γ intensity is given by

$$\varepsilon'_\gamma = \frac{AREA}{I\gamma}$$

where AREA is the transition peak area.

The gamma transitions intensities and energies considered in our calculations were the Bellman's[19] values for the nitrogen and the Kennett's[25] values for the chlorine, while for the chromium isotopes the data came from the Nuclear Data Sheets[26,27,28].

RESULTS

The peak area calculations were done by using the GAUSSV[29] and SAMPO[30] computer programs.

First we determined the nitrogen concentration of a National Bureau of Standards target of KNO_3(SRM 193) with a known percent nitrogen concentration of 13.85 ± 0.01.

With this measurement we were able to test all the experimental conditions and also the mathematical method of analysis. We could also verify the nickel standard(geometry and dimension), the sample holder material and the relative efficiency values.

The result shown a nitrogen percent content of 13.85± 0.38, which allows us to say that the experimental conditions and the method used for the nitrogen determination are reliables.

The several seeds selected for analysis by means of the thermal neutron capture prompt gamma-rays technique are soybean, common bean, peas and rice. We selected this particular set chiefly because, as shown in Table I, the nitrogen content varies from a low value for rice to a high value for soybean, allowing a better evaluation of the technique application performance.

Other reasons for our choice were motivated by the fact that the rice and the common bean form the main brazilian nourishment, and the soybean, for its large protein content, is an important substitute for animal protein.

The results of the nitrogen determination in seeds using eq.5 are presented in Table II.

TABLE I

Typical Values of Nitrogen Percent Content in Grains

Grain	Rice[32]	Common Bean[32]	Peas[33]	Soybean[33]
%N	0.9 - 2.1	2.9 - 4.8	2.9 - 4.8	5.9 - 8.0

TABLE II

Percent Nitrogen Measured in Several Samples

Seed \ Sample nº	1	2	3	4
Soybean	6.34 ± 0,16	6.11 ± 0.28 6.13 ± 0.18	6.22 ± 0.32	6.28 ± 0.18 6.23 ± 0.29 6.27 ± 0.18
Common Bean	3.43 ± 0.20	3.49 ± 0.20		
Peas	3.60 ± 0.11	3.69 ± 0.10		
Rice	1.02 ± 0.09			

The reproducibility of the method was tested by measuring several times some soybean samples and the results show an 0.8% deviation. This also allows us to say that there is not nitrogen loss during the irradiation.

DISCUSSION

The thermal neutron capture prompt gamma-rays technique shown a good performance for the nitrogen content determination in seeds. Although we used a research reactor as neutron source, the main characteristic of the method is its versatility, since its permits the utilization of an isotropic neutron source. Despite the fact that the isotropic neutron source flux is lower than the reactor flux, we can reduce the solid angle factor by approximating the detector to the sample.

Since the neutron flux from an isotropic neutron source is constant, a standard is not required, simplyfing the sample preparation.

Furthermore, as the 10.83 ^{15}N transition is unique in that energy region, it is possible to use a sodium iodide for detecting the gamma-rays, at a lower cost than with a Ge(Li) detector, also facilitating the in situ analysis of the seeds.

Using an isotropic neutron source, the gamma dose for the seed sample is very low, so the analysis is also non-destructive in the genetic sense, as the embryo dose cannot exceds about 100 rads[4]. This is very interesting for the plant breeding programmes, because the seeds with higher protein content can be used in a second generation.

The advantage of using the prompt gamma-ray technique in the plant breeding programmes lies also in the possibility of the continuous measurement of N concentration, which would allow automatic determination of the protein content. Using a NaI(Tl) detector, the instrumental requirements are comparatively low, so it seems to be no severe limitations on the future application of this technique.

REFERENCES

1. PIMENTEL,D. et alli, Science, 190, 754-761 (1975).
2. PARPIA,H.A.B., Improving Plant Protein by Nuclear Techniques(Proc.Symp. Vienna,1970), IAEA, Vienna, 3-12(1970)
3. KAUL,A.K., Nuclear Techniques for Seed Protein Improvement(Proc.Meeting Neuherberg,1972), IAEA, Vienna, 1-106 (1973).
4. NIEMANN,E.G., At.Energy Rev., 18, 125-170 (1980).
5. INTERNATIONAL ATOMIC ENERGY AGENCY, Breeding for Seed Protein Improvement Using Nuclear Techniques(Proc. Meeting Ibadan,1973), IAEA, Vienna, 181-186(1975).
6. INTERNATIONAL ATOMIC ENERGY AGENCY, Breeding for Seed Protein Improvement Using Nuclear Techniques(Proc. Meeting Ibadan,1973), IAEA, Vienna,(1975), Annex III.
7. INTERNATIONAL ATOMIC ENERGY AGENCY, Nuclear Techniques for Seed Protein Improvement (Proc.Meeting Neuherberg, 1972), IAEA, Vienna, 411-415, (1973).
8. BRUNE,D. & ARROYO,A., Anal.Chim. Acta, 56, 473-476(1971).
9. SRAPENYANTS,R.A. & SAVELIEV,I.B., J.Radioanal.Chem., 38, 247-255(1975).
10. KOSTA,L.; RAVNIK,V.; DUMANOVIĆ,J., New Approaches to Breeding for Improved Plant Protein(Proc.Panel Röstanga, 1968), IAEA, Vienna, 161-168 (1969).
11. BRUNE,D. et alli, Int.J.Appl.Radiat.Isot., 24, 1-6(1973).
12. NIEMANN,E.G. & NEUMULLER,D., Nuclear Techniques for Seed Protein Improvement(Proc.Meeting Neuherberg,1972), IAEA, Vienna, 339-346 (1973).
13. RUPNIK,P. et alli, Nucl.Instrum.Meth., 142, 209-211 (1977).
14. JOHANSSON,A. et alli, New Approaches to Breeding for Improved Plant Protein(Proc.Panel Röstanga,1968), IAEA, Vienna, 173-184 (1969).
15. ANDRÁS,L. et alli, Radiochem.Radioanal.Letters, 40, 27-36 (1979).
16. TIWARI,P.N., Radiochem.Radioanal.Letters, 6, 363-370 (1971).
17. TIWARI,P.N., Int.J.Appl.Radiat.Isot., 22, 587-592(1971).
18. BELLMANN,D., In AJZENBERG-SELOVE,F., Nucl.Phys.A, 360, 132 (1981).
19. ISLAM,M.A., PRESTWICH,W.V., KENNETT,T.J., Nucl.Instrum. Meth., 188, 243-245 (1981).
20. PECEQUILO,B.R.S. et alli, CONF-800433, 247-257 (1980).
21. PECEQUILO,B.R.S., PhD. Thesis, IEA-DT-078 (1977).
22. ISHAQ,A.F.M., Z.Physik A, 281, 365-372 (1977).
23. CARBONARI,A.W., MsC. Thesis, IPEN-CNEN/SP, 1983.

24. CHART of the Nuclides, Knolls Atomic Power Laboratory (1968).
25. KEMMET,T,J,, ISLAM,M.A., PRESTWICH,W.V., Can.J.Phys.59, 93-108 (1981).
26. NUCLEAR Data Sheets, 23, 208 (1978).
27. NUCLEAR Data Sheets, 21, 349 (1977).
28. NUCLEAR Data Sheets, 23, 487 (1978).
29. HELMER,R.G. & PUTMAN,M.H., AMCR-1043 (1972).
30. ROUTTI,J.T., UCRL-19452, (1969).
31. HARTWIG,E.E., New Approaches to Breeding for Improved Plant Protein(Proc.Panel Röstanga,1968), IAEA, Vienna, 67-70 (1969).
32. INTERNATIONAL ATOMIC ENERGY AGENCY, Breeding and Selection Methods for Seed Protein Improvement (Proc. Meeting Baden, Austria, 1977. Proc.Meeting Vienna,1977), IAEA, Vienna, 411-420 (1978).
33. EGGUM,B.O. New Approaches to Breeding for Improved Plant Protein (Proc.Panel Röstanga, 1968), IAEA, Vienna, 125-135 (1969).