Study of nuclear reaction method for the determination of protein content in wheat seeds

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Abstract A 13.4 MeV deuteron beam from the 1.2 meter cyclotron of Sichuan University was used to determine the protein content in wheat seeds on the basis of (d,p) reactions. The influence of the variation of the water content in seeds has been investigated.
Key words Nuclear reaction analysis, Protein content, $^{14}N/^{12}C$ ratio
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1 Introduction

Protein is important for human health.The quantity and quality of protein supplied by plant material is the most important basis for human and animal nutrition. One of the most promising ways to reduce the protein deficiency in developing countries without interfering with the eating habits of the population is to increase the protein content and to improve the protein quality of the staple food, such as the cereals and legumes. Plant breeding programmes directed towards this goal are confronted with the need for protein screening.[1,2] The first step will always be the protein determination in seeds.

As the protein content is strongly correlated to the nitrogen content, methods to measure nitrogen can be used to estimate the protein content.[3] The standard method for the determination of protein content in plant sample is the Kjeldahl chemical analysis method. But Kjeldahl method is time-consuming, destructive and involved in problems of environmental contamination by wastes. So a number of nuclear methods have been suggested to meet the requirements in practical screening.[4]

The aim of this work was to establish an easy and reliable screening method for protein by using nuclear reactions induced by 13.4 MeV deuterons produced by the 1.2 meter cyclotron of Sichuan University.

2 Experimental method and procedure

Grain seeds are composed of four major elements: carbon, oxygen, hydrogen and nitrogen. Nitrogen is mainly contained in protein and the content of nitrogen in various kinds of protein is nearly the same. So the nitrogen concentration is closely related to the protein content of seeds. For wheat, the protein content can be obtained through the nitrogen content multiplied by a costant of 5.70. As the carbon content in the major organic matters of seed (protein, cellulose, starch, sugar, etc.) is about the same, carbon may serve as an indicator for dry organic matter. So the ratio of $^{14}N/^{12}C$ may indicate the content of protein in seeds. As the water content in seeds directly influences the concentration of oxygen in seeds, the content of oxygen or its variation may indicate the change of the water content in seeds.

When the grain sample was impacted by deuteron beam, various (d,p) reactions were induced. The reaction *Q*-values are listed in Table 1. Due to the large positive *Q*-value of $^{14}N(d,p)^{15}N$, it is easy to choose the signals from the reaction of ^{14}N by energy

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discrimination in an emitted charged particle energy spectrum.

Table 1 The Q value for various (d,p) reactions

Reacting nucleus	^{12}C	^{13}C	^{14}N	^{15}N	^{16}O
Q value (MeV)	2.72	5.95	8.61	0.26	1.92

For $^{14}N(d,p)^{15}N$ reaction induced by 13.4 MeV deuterons, the differential cross section increases with the increase of emitted proton angle. And for $^{12}C(d,p)^{13}C$ reaction, the maximum differential cross section appears at the emitted proton angle of 120°. On the other hand, just at this angle, the minimum of the differential cross section for $^{16}O(d,p)^{17}O$ reaction appears. By using the characters of these reactions, the experimental conditions could be optimized.

The experimental setup is shown in Fig.1. Grain samples were placed in a vaccum chamber. The samples were automatically moved into the beam position by a stepping motor and irradiated by 13.4 MeV deuterons produced by the 1.2 meter cyclotron of Sichuan University. The charged particles produced were recorded by two CsI detectors which possess the characters of good endurance for irradiation, long-term stability and easy calibration. One CsI detector was placed at the maximal angle (165° with respect to the incident deuterons) allowed by the experimental condition for the detection of proton from the $^{14}N(d,p)^{15}N$ reaction, and the other was placed at the angle of 120° to record the proton mainly from the $^{12}C(d,p)^{13}C$ reaction and reduce the influence of oxygen at most. In front of each detector a suitable Cu foil was used to stop the scattering deuterons and the alpha particles from the (d,α) reactions. The diameter of the CsI scintillator was 2 cm and the distance between the sample and the detector was 13.5 cm.

During the experiment, a standard foil sample of polyimides ($C_{22}H_{14}N_2O_5$, 20μm in thickness) was used to calibrate the detector system. First the MCA (multi-channel analyzer) was used to record the charged particle spectrum from the standard foil sample irradiated by 13.4 MeV deuterons. The peak channels of proton from nitrogen and carbon could be determined and the thresholds of the SCA could be set. When the wheat samples were analysed, the counts of the two counters need to record simultaneously.

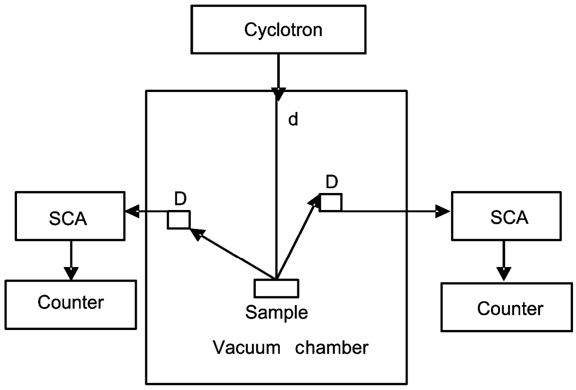

Fig.1 Experimental setup.
D: detector and preamplifier. SCA: single channel analyzer.

3 Results and discussion

In this experiment, wheat seeds of four varieties were analysed. The results were compared with those obtained by using Kjeldahl method. A strong positive correlation was demonstrated between the ratios of $^{14}N/^{12}C$ and the Kjeldahl values (Fig.2) and thus the nuclear reaction method provides a good estimate of nitrogen content of the wheat. During the process of wheat analysis, only the counts of the counters need to record. It is easy to automate the measurement and high analysis rate could be achieved.

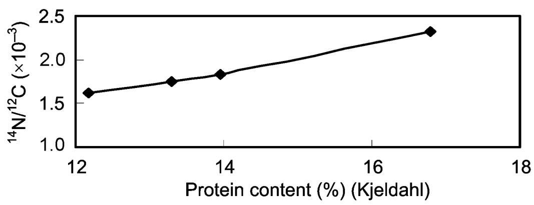

Fig.2 A comparison of $^{14}N/^{12}C$ ratios with the Kjeldahl values.

As the energy of proton from $^{12}C(d,p)^{13}C$ reaction induced by 13.4 MeV deuterons is almost the same as that from $^{16}O(d,p)^{17}O$ reaction, the measurement of carbon content used in this experiment was influenced by the oxygen content. Especially in practical selection of wheat, the water content in different samples may vary greatly. So the change of the oxygen content caused by water should be monitored. And relational correction should be made. Note that the maximum of differential cross section for $^{12}C(d,p)^{13}C$ reaction induced by 13.4 MeV deuteron appears at 120° and the minimum at 150°. On the other hand, the minimum of the differential cross section for $^{16}O(d,p)^{17}O$ reaction appears just at 120°. So there is a maximal difference of proton contribution for $^{12}C(d,p)^{13}C$ and $^{16}O(d,p)^{17}O$ reactions between angles of 120° and 150°. The ratio of proton counts recorded at 120° to those recorded at 150° may indicate the change of the oxygen content.

The samples with the same element composition as wheat but with different C/O mass ratios were prepared by using the mixture of oxalic acid ($C_2H_2O_4$•$2H_2O$), lactose ($C_{12}H_{22}O_{11}$•H_2O), melamine ($C_3H_6N_6$), flour and pure carbon powder. Experimental results demonstrated that the ratios of N(120°)/N(150°) were sensitive to the mass ratios of C/O (Fig.3). Here N(120°) represents the proton counts recorded by the detector which was placed at the angle of 120° and 13.5 cm from the sample, while N(150°) is the proton counts simultaneously recorded by the detector which was placed at the angle of 150° and 13.5 cm from the sample. The change of the C/O mass ratio in wheat seeds may be caused by the change of the protein content besides caused by the water content. But the increase of the nitrogen content in grain always accompanies the decrease of the oxygen content. The summation of the nitrogen content and the oxygen content changes little with the variation of the protein content in seeds. And for thick wheat sample, if the summation of the nitrogen content and the oxygen content is the same, the proton contribution caused by 13.4 MeV deuterons is nearly the same at the angles of 120° and 150°. So the ratios of N(120°)/N(150°) could be used to represent the change of the water content in grain samples. And the relational correction could be made accordingly.

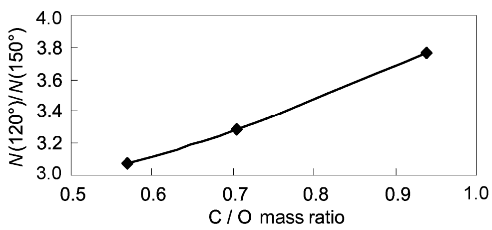

Fig.3 N(120°)/N(150°) ratios versus C/O mass ratios.

The deuteron current used in this experiment was about 0.2 nA and the exposure time was several minutes per sample. This time can easily be reduced to several seconds by increasing the solid angles, the number of detectors and the beam current. The error of the protein content determined by Kjeldahl chemical analysis method was less than 1% and the statistical uncertainty for the proton counts recorded by CsI-detector was also less than 1%.

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