DETERMINATION OF THE SULFUR TO NITROGEN RATIO IN CEREAL GRAINS BY NEUTRON ACTIVATION ANALYSIS

by

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Approved by:

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To my wife, Gail, and to my Father and Mother.

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INTRODUCTION

The importance of the sulfur to nitrogen (S:N) ratio in diets has been studied since 1963. Miller and Donoso (37) reported that the value of the protein in most human diets is limited by the content of sulfur-containing amino acids. An estimation of the amount of these amino acids was made by chemical determination of the S:N ratio in the diet. Correlating these S:N values with the results of feeding these diets to rats, the authors proposed an empirical function, %S/%N X 1000, to represent the value of protein in mixed diets. Thus, the protein nutritive value of a mixed diet could be determined by chemical analysis on the diet. Other studies of the dietary value of protein have been made (38,43). Pellett, Kantarjian, and Jamalian (41) reported that the S:N formula used by Miller and Donoso could not be applied to Middle Eastern diets. Nevertheless, it can be seen that the S:N ratio in human diets and the chemical determination of S:N ratio in foods is of considerable interest.

For a study of animal diets, the animals must be separated into two groups: simple-stomach and ruminants. For animals with simple-stomachs (pigs, chickens, rats, etc.) on diets of cereal grains, lysine has been found to be the amino acid limiting the nutritive value of the diet (15). Lysine can be supplied in sufficient amounts by supplementing the diet with soybean meal. In the lysine-sufficient diet, methionine and/or cystine become the amino acids limiting the nutritive value of the diet. Methionine and cystine are the sulfur-containing amino acids in cereal grains. It can be seen, then, that knowledge of the S:N ratio in cereal grains would give an indication of the nutritive value of the protein in the cereal grain.

Ruminant animals present a different situation. Bacteria in the rumen convert non-amino acid sulfur to a form usable by the animal (15). Thus the sulfur supplied in ruminant animal diets need not be a sulfur-containing amino acid. Still, researchers have found (15) that a S:N ratio of approximately 1:15 in the diet of ruminants gave maximum nutritive value to ingested proteins.

The S:N ratio has been found of considerable value to agronomists. The quantity of cystine and methionine in plants and cereal grains has been shown to be directly related to the availability of sulfur to the growing plant (6,50,54). The S:N ratio has been used to detect sulfur deficiencies in plants (3). More recently, a problem has developed with the increasing emphasis on high rates of application of nitrogen fertilizers to increase yields. It has been found that when the S:N ratio becomes too small, the nitrogen is stored in the plant as non-protein nitrogen (e.g. nitrates) (53). In sulfur-sufficient soils, S:N ratio of approximately 1:19, both the quantity of dry matter and the percent of sulfur-containing amino acids increased (54).

It can be predicted from this discussion that the S:N ratio will become of much greater interest in the areas of plant, animal, and human nutrition in the near future.

The development of a technique to determine sulfur and nitrogen in cereal grains rapidly and easily would simplify classification of cereal grains according to their nutritive value. Such a technique could hopefully be engineered to an on-line system in places where large quantities of cereal grains are handled or used, such as flour mills and grain elevators.

A physically non-destructive method would be desirable to speed the analysis. Also, if no genetic damage were inflicted on the germ, geneticists and plant breeders could use the proposed method to analyze small amounts of

sample and use the sample again.

To analyze non-destructively requires a technique capable of analysis in the presence of large numbers and amounts of interferences such as are found in cereal grain samples (46). Neutron activation analysis seemed to offer the greatest probability of fulfilling the demands of such an analytical technique.

LITERATURE REVIEW

The determination of nitrogen in biological materials by the Kjeldahl method has long been the standard method for nitrogen analysis in cereal grains (4). This method is based on a sulfuric acid oxidation of the organic material to convert the nitrogen into ammonium sulfate. The ammonium sulfate is decomposed to ammonia and distilled into standard acid after which the acid is back-titrated to determine the amount of ammonia produced. The greatest drawback of this procedure in its application to the proposed project was the time involved for the oxidation step (two to three hours).

Eckhoff (17) and Wood (53) applied fast neutron (14.1 MeV) activation to determine nitrogen in cereal grains. This method is based on the $^{14}N(n,2n)^{13}N$ reaction. The half-life of ^{13}N is 9.96 minutes. The factors that made this method very attractive to the proposed problem were the speed (total time approximately 20 minutes) and the non-destructive nature of the analysis.

The determination of sulfur in samples containing large numbers and/or large quantities of interfering elements has been a long-standing problem. The problem was initially approached with a gravimetric determination of sulfur after precipitation as barium sulfate from large samples (1,21,22). Application of this method is beset by several disadvantages. The most serious disadvantages include the large volume of sample required, the amount of time involved, and the co-precipitation of interfering ions with the $BaSO_4$.

A turbidimetric technique has been developed based on the precipitation of sulfate with $BaCl_2$ in the presence of various dispersing agents to keep the resulting $BaSO_4$ from settling (11,34,51). The reduction of intensity of a light beam when passed through these suspensions was used to determine the amount of $BaSO_4$ present. The disadvantages of this method are numerous.

For instance, Butters and Chenery (11) reported that soil and plant oxidations required overnight digestions. In addition, a 1.5 hour standing time was required before the measurement was taken. The same authors also reported that the size of the $BaCl_2$ crystals is critical to the reproducibility of the analysis, so that new standard curves must be made for each new batch of $BaCl_2$ crystals used. In spite of these precautions, the authors reported that occasional determinations were still in error by more than 10%. Steinbergs (51) found that the sensitivity and reproducibility of the turbidimetric analysis for sulfate depended on the presence of insoluble impurities in the $BaCl_2$ solution. He used "$BaSO_4$-seeded" $BaCl_2$ suspensions to determine sulfate.

A nephelometric technique was developed by Martin and Stephen (33). A nephelometric determination was made by measuring the light scattered out of a light beam by small particles in a solution which was placed in the light beam. The authors used 4-amino-4'-chlorodiphenyl hydrochloride reagent. They reported that this reagent was unstable and needed to be prepared, standardized, and used the same day. This technique was also used by Mendes-Bezerra and Uden and applied to organic samples and hydrocarbon oils (36).

X-ray fluorescence has been applied to the determination of sulfur (2,10). Brown and Kanaris-Sotirion (10) applied this method to soils and reported an analysis time of four minutes per sample. However, these were mineral soils which did not have the interference problems associated with organic samples. The same authors applied this method to organic soils but did not give an analysis time per sample. The assumption may be made that the time was considerably longer since the organic soil samples were heated at 450°C to determine weight loss from oxidation of organic material.

The sulfur-analysis method that has become the most widely used is the methylene-blue method of Johnson and Nishita (31). They developed this method

to estimate the sulfate-sulfur content in plant material, soils, and irrigation waters. The sulfate was reduced to hydrogen sulfide and trapped in a solution of zinc acetate. Para-amino-dimethylamine was added to develop the deep blue color of methylene-blue. The absorbancy of the solution was measured at 670 mμ. The authors reported that nitrate was a serious interference if it carried over to the developing solution. There are a few disadvantages to this method as an application to the proposed project. Amounts of sulfur greater than 50 μg yield methylene-blue solutions that deviate from linearity. Very exacting chemical pretreatment of the apparatus is required. All traces of sulfur have to be removed from the lubricating grease and from the rubber stoppers. It was also noted that the first few runs would give low results due to removal of some sulfur by the glassware. The molar absorbance for the methylene-blue solution, calculated from the average absorbancies at 670 mμ over the range of 5-50 μg of sulfur, was reported to be 34,500. The reported time of analysis was two hours.

This method has also been used to determine total sulfur after preliminary oxidation of sulfur to sulfate (29,32,42,52). Gustafsson (27), and Dean (13) discussed more of the problems associated with the methylene-blue method.

Other colorimetric methods have also been developed. Bertolacini and Barney (7) precipitated $BaSO_4$ with barium chloroanilate and measured the resulting acid chloroanilate colored solution at 530 mμ. Cations interfered but were removed by ion exchange. Dean (13) prepared a colloidal suspension of bismuth(III) sulfide and colorimetrically determined the amount of sulfur. Bloomfield (9) analyzed soils for total sulfur by converting the sulfur to sulfur dioxide and absorbing it in a sodium tetrachloromercurate solution. The sulfur determination was made colorimetrically with p-rosaniline and

formaldehyde. They reported that the method was linear to a concentration of 10 μg of sulfur in 50 ml of solution.

In all of these colorimetric methods, special precautions were required in the preparation and handling of reagents and samples. Blanks must be run with each sample, and new standardization curves must be made with each new batch of reagents. Coupling these factors with the length of time required to oxidize a solid grain sample, we evaluated these colorimetric methods as unsuitable for this project.

Other common methods for the determination of sulfur come under the general heading of titrimetric techniques. Gillespie (25) passed aqueous sulfate solutions through an ion exchange column and titrated the mineral acid that was formed. Siegfriedt et al., (48) titrated sulfate solutions with barium chloride with tetrahydroxyquinone as an indicator. Jenkinson (30) used barium perchlorate to titrate sulfate, detecting the end point with Sulphonazo III indicator. Bird and Fountain (8), and Soep and Demoen (49) reduced the sulfate to sulfide and titrated with mercury(II) acetate and lead(II) nitrate, respectively, using a dithiozone-acetone indicator. Dixon (16) utilized a potentiometric titration of silver from silver sulfide to determine the amount of sulfide trapped from a gas stream. In titrimetric determinations, the system must be relatively clear of most elements since many interfere (48). This requires rather lengthy chemical pre-treatment on complex systems (16,30). This method has also been used for sulfur detection in relatively clean systems (49). There is also some indication that an experienced technician is required to produce reproducible results (8,48).

A fluorimetric technique was developed very recently by Axelrod et al. (5). The HCHO-bisulfite reacts with a fluorescent molecule with the formation of a complex species which does not fluoresce. Thus, from a knowledge of the

concentration of fluorescent material initially present, the amount of sulfur was determined. The authors used this as a method of sulfur dioxide analysis.

A method based on flame spectrophotometry has been developed for the indirect determination of sulfur. Shaw (47) used a standard barium chloride solution to precipitate the sulfate formed from oxidized biological samples. He then used flame spectrophotometry to determine the excess barium. Gersonde (24) precipitated the sulfate as strontium sulfate and then determined strontium by flame spectrophotometry.

A considerable amount of work has been done to date in an attempt to analyze for sulfur by neutron activation analysis. The $^{34}S(n,\gamma)^{35}S$ reaction has been utilized by Ross (44) and Niese (40). Sulfur-35 is a weak beta emitter, and rigorous precautions must be taken to clear the sample of all other beta emitters before the sulfur-35 activity can be detected. The other obvious drawback to this method is the long half-life of sulfur-35 ($t_{\frac{1}{2}}$ = 87 days). This makes the sulfur-35 method very slow and tedious.

McCandless (35) irradiated algal polysaccharides with a fast neutron flux and observed the $^{32}S(n,p)^{32}P$ reaction. Phosphorus interferes by the $^{31}P(n,\gamma)^{32}P$ reaction. The author suggested shielding the sample with cadmium to remove the thermal neutron flux and thus eliminate the phosphorus interference. This suggestion has proved impractical in the present work (see Experimental).

Fujii et al., (23) noted in their fast neutron work that for sulfur, no prominent gamma ray photopeaks were found that would be suitable for the neutron activation analysis under experimental conditions.

Debrun and Albert (14) reported that unsatisfactory results and interferences were met in the determination of sulfur by neutron activation analysis. These authors determined sulfur by the charged particle reaction $^{35}S(p,d)^{34m}S$.

Elejalde and Albisu (19) determined sulfur in petroleum products by the $^{36}S(n,\gamma)^{37}S$ reaction. They reported an accuracy of ±0.15% sulfur.

Very recently, Dams and co-workers used the $^{36}S(n,\gamma)^{37}S$ reaction to determine sulfur in air pollution studies. They reported a detection limit of 25 μg and found the method quite satisfactory for their work.

Taking into consideration the interfering materials known to be in cereal grains and the difficulties associated with sulfur neutron activation analysis reported in the literature, we concluded that careful attention would have to be given to background counting and photopeak resolution.

EXPERIMENTAL

The problem was approached with the idea of establishing a reliable method to use as a standard for the analysis of sulfur in cereal grains, even though this might require a destructive technique. This standard technique would be the reference for further development of techniques to include fast neutrons and/or non-destructive analyses.

The initial approach to analyze sulfur in cereal grains was to use the $^{36}S(n,\gamma)^{37}S$ reaction. Sulfur-37 has a 3101.55 KeV gamma ray (20) and a half-life of 5.1 minutes. Calcium interferes with this reaction by $^{48}Ca(n,\gamma)^{49}Ca$. Calcium-49 has a gamma ray energy of 3084.49 KeV and a half-life of 8.8 minutes. The NaI(Tl) crystal used to detect the radiation could not resolve the small difference in gamma ray energies. From a knowledge of the respective thermal neutron cross sections, isotopic abundancies, and average abundancies of sulfur and calcium in wheat, it was predicted that the calcium-49 activity would be about fifteen times greater than the activity of sulfur-37.

An attempt was made to chemically separate calcium and sulfur after activation. The wheat sample (approximately 0.5 gram) was oxidized by an $HF \cdot HNO_3$ concentrated acid mixture. The more commonly used $HClO_4 \cdot HNO_3$ oxidation was not acceptable due to the $^{37}Cl(n,p)^{37}S$ interference reaction.

The procedure was as follows. Approximately 20 ml of concentrated AR Grade HNO_3 (Mallinckrodt Chemical Works) was added to approximately 0.5 gram of wheat in a polyethylene beaker (Tri-Pour Disposable Beakers, Sherwood Medical Industries Inc.) along with three or four drops of Reagent Grade 48% HF (Fisher Scientific Company). The solution was heated in a water bath until the wheat was completely oxidized to form a clear yellow solution. This oxidation took about twenty minutes to complete.

About 10 ml of saturated solution of AR Grade boric acid was added to the oxidized wheat sample to tie up any free fluoride ions.

The sample was placed in a polyethylene vial (Olympic Plastics) which was placed in a polystyrene vial and inserted in the rotary specimen rack, RSR, in the Kansas State University Triga Mark II Reactor*. It was irradiated at 200 kW of power for 10 to 15 minutes.

The solution was then neutralized with concentrated AR Grade ammonium hydroxide (Mallinckrodt Chemical Works). Inert calcium ion, AR Grade $Ca(NO_3)_2$, and oxalate ion, AR Grade $(NH_4)_2C_2O_4$, were added to scavenge the calcium-49 out of solution. The calcium scavenge was repeated twice. Then inert barium ion, AR Grade $BaCl_2$, and sulfate ion, dilute AR Grade H_2SO_4, were added and the barium sulfate was centrifuged to separate it from the remaining solution. This $BaSO_4$ was counted with a 3x3 inch NaI(Tl) crystal and a TMC 4096 (Technical Measurements Corporation) multiparameter pulse-height analyzer*. The sample was counted for a five minute period and then immediately counted for another five minute period. This was continued to obtain a time decay of the activity in the energy region of 3.1 MeV. The activity was plotted on semi-logarithmic paper and an attempt was made to resolve the decay curve into the individual components. It was very evident that more than just sulfur-37 was precipitated with the $BaSO_4$, since activity from species with half-lives greater than 5.1 minutes was observed. The 5.1 minute sulfur-37 activity was observed, but the activity was so low that no values of any statistical significance were obtained.

Several interesting characteristics were noted after the oxidized wheat solution was neutralized with ammonium hydroxide. Some turbidity occurred in

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the solution which appeared to be a gelatinous-type of precipitate. Subsequent centrifugation gave a layered precipitate, one layer crystalline and one layer gelatinous. It was not possible to completely centrifuge all the insoluble particles out of solution. These precipitates were not soluble in water or strong acid. No attempt was made to analyze the precipitates except to check, by neutron activation analysis, whether they contained any sulfur or calcium. The results were negative.

The oxidized wheat solution turned a turbid black upon standing after being neutralized. Again, no attempt was made to analyze the black turbid material. It was noted in later work that the oxidized wheat solution was a self-indicator of pH. The solution would turn from a pale yellow at pH<1.0 to a deep copper at pH>9.0. The solution would then turn black upon standing at high pH.

An attempt was made to remove calcium ion from solution by ion exchange. A strong acid, RSO_3^-, cation exchange column in the hydrogen ion form, Rexyn 101 (H) Research Grade (Fisher Scientific Company) was made. A piece of vinyl tubing (Fisher Scientific Company) was used for the column. Vinyl tubing was used instead of glass to eliminate the contamination of the sample with calcium leached out of the glass. The column was 4-6 cm high and 7 mm i.d. The oxidized wheat sample was neutralized and passed through the column. The column was washed twice with 10 ml of H_2O, and the washings were added to the sample. The volume of sample was reduced to about 7 ml by heating on a hot water bath. The sample was placed in a polyethylene vial which was placed in a polystyrene irradiating vial and irradiated in the RSR of the KSU Triga Mark II reactor for 15 minutes at 200 kW of power. The sample was counted immediately after a transfer time of two minutes on the NaI(Tl) system described above.

Initially the results looked promising. The photopeak in the 3.1 MeV region, upon subtraction of the background, decayed with a 5-6 minute half-life. Two observations produced questions whether sulfur-37 or calcium-49 was being observed. A least-squares analysis of the data produced a half-life of 7.2 minutes which is about midway between the half-lives of sulfur-37 and calcium-49. Also, calculations based on the assumption that the activity was all sulfur-37, gave a value for sulfur of twenty times the amount expected in wheat (39). This is uncomfortably close to the difference in the expected activities of calcium-49 in relation to the activity of sulfur-37. The question also arose whether the oxidized wheat solution caused a breakdown of the RSO_3^- resin, thus releasing sulfur into the system. An attempt was made to use a weak cation exchange resin, $RCOO^-$. It was found that this resin did not remove any calcium ion.

A 30 cc Ge(Li) semiconductor detector* was made available for this project. This detector had a resolution of approximately 5-6 KeV full width at half maximum on the 1332 KeV cobalt-60 photopeak. It was shown that this system could resolve the calcium-49 and sulfur-37 photopeaks up to a certain point depending on the ratio of calcium to sulfur and on the quantity and type of interfering elements. An attempt was made to analyze, non-destructively, a chickpea sample which previously had been analyzed for methionine and cystine (15). The calcium-49 and sulfur-37 photopeaks were not resolved.

The fast neutron reaction $^{34}S(n,p)^{34}P$ seemed to present a possible method for fast sulfur analysis. Phosphorus-34 has a half-life of 12.4 seconds and a 2.10 MeV gamma ray (26). A whole wheat sample was placed in a cadmium-shielded capsule and irradiated for one minute in the pneumatic transfer

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system, PTS, of the KSU Triga Mark II reactor. The capsule was cadmium lined to essentially remove the thermal neutron flux reaching the sample. This was expected to reduce the number of interfering n,γ reactions and the $^{31}P(n,\gamma)^{32}P$ reaction in particular. The PTS was used as a rapid transfer system to move the sample from the reactor core to the counter in the shortest possible time, theoretically four seconds. The sample was counted with a 3x3 inch NaI(Tl) crystal and a 100 channel analyzer. It was noted from the time-decay plot that there was some short-lived activity, but it was impossible to acertain whether or not it was phosphorus-34. A four gram sample of NF Grade (sublimed) sulfur (Fisher Scientific Company) was irradiated for one minute in the PTS and counted in the same manner as the wheat sample. A phosphorus-34 peak was very evident. Calculations were made to estimate the level of activity of phosphorus-34 to be expected from a wheat sample containing 2000 ppm sulfur (39). It was found that 18 counts per minute would be the maximum expected, much too low to be statistically significant.

The $^{32}S(n,p)^{32}P$ reaction was utilized in an attempt to analyze for sulfur in wheat. Phosphorus-32 has a 14.7 day half-life and is a strong beta emitter (26). When counted with a scintillation detector, the radiation from phosphorus-32 appeared as Bremsstrahlung. The greatest interference with this method was the $^{31}P(n,\gamma)^{32}P$ reaction. McCandless (35) suggested that the $^{31}P(n,\gamma)^{32}P$ reaction could be eliminated by shielding the sample with cadmium. Samples of wheat, sulfur, $(NH_4)_2HPO_4$ for a phosphorus reference, and NH_4Cl for a chlorine reference were placed in a polystyrene vial that was completely lined with cadmium. The polystyrene vial was placed in the RSR of the reactor and irradiated for fifteen minutes on one set of samples and for one hour on another set of samples at 250 kW of power. The phosphorus-32 produced in each sample was counted with a NaI(Tl) crystal detector with a 100 channel

analyzer. The decay of the activity was followed to prove the activity was phosphorus-32 only. A calculation was made to estimate the activity expected in wheat from the $^{31}P(n,\gamma)^{32}P$ reaction, based on the knowledge of the activity in the phosphorus reference and an average value of phosphorus in wheat of 4000 ppm (45,46). It was determined that all of the observed phosphorus-32 activity in the wheat sample was from the $^{31}P(n,\gamma)^{32}P$ reaction, indicating that this reaction was occurring unabated behind the cadmium shield. It was noted (28) that the neutron cross section for the $^{31}P(n,\gamma)^{32}P$ reaction is essentially linear up to 300 eV which is well beyond the cadmium cross section resonance cutoff of about 1.4 eV. Therefore, cadmium shielding of a sample did not reduce the $^{31}P(n,\gamma)^{32}P$ reaction.

An attempt was made to detect the $^{34}S(n,p)^{34}P$ reaction by activation with 14.1 MeV neutrons. The interference in this method was from the nitrogen-16 which was produced by the $^{16}O(n,p)^{16}N$ reaction. Nitrogen-16 has a half-life of 7.1 seconds. The gamma energy of ^{16}N is so large (7.1 MeV) that a significant Compton continuum resulted in the NaI(Tl) detector. This Compton continuum in all channels below the 7.1 MeV upper limit made resolution of any photopeaks of lower energy and similar activity impossible. A sulfur reference sample was irradiated with the wheat sample. Calculations were made from the activity induced in the sulfur reference. It was found that a total activity of approximately 0.5 counts would be expected from a one gram sample of wheat.

Attention was returned to the ion exchange work done previously. The Ge(Li) detector was used to collect the data. A calcium reference as AR Grade $CaCO_3$, and a sulfur reference were irradiated with the sample of wheat or chickpeas. It appeared that the photopeak from the grain sample was in the same channel as the calcium-49 photopeak from the reference. One run was made in which the irradiated wheat sample was passed through the strong acid cation

exchange column. The column was washed with two 10 ml portions of H_2O. These washings were also counted. No calcium-49 was found in the washings. It was found that no calcium was held back on the column. All of the calcium passed through the column immediately and was detected in the sample portion of the effluent. Also, a calculation of the calcium level in the wheat from the activity in the 3.1 MeV photopeak and the activity induced in the calcium reference, gave values similar to those obtained by other methods (45,46). Apparently the calcium was held in some form or another, perhaps a chelate, in the oxidized wheat sample and was evidently not "free" to react with the strong acid cation exchange resin.

A calculation was made to obtain a value of the expected activity from a 0.5 gram wheat sample due to the sulfur-37. The calculation was made from the known activity induced in a sulfur reference and the average sulfur content of wheat. The value was found to be 3-5 counts per minute. A whole chickpea sample, 0.5 grams, was irradiated and counted on the Ge(Li) system which was set to optimize the resolution of the sulfur-37 and the calcium-49 photopeaks. One of the problems that had been encountered when counting the entire spectrum of a whole grain sample was the very high dead time factor, greater than 20%, on the analyzer. To optimize the system for counting a whole grain sample, the base line of the amplifier was raised to eliminate the activity below an arbitrarily chosen level of 1.5 MeV. This eliminated from the analyzer the intense activity from manganese-56, and also lowered the dead time to less than 2%.

The active chickpea sample was counted and an attempt was made to resolve the low level of activity that had been calculated for sulfur-37. The results, although statistically poor, showed promise. The final procedure was based on these results.

Samples of whole chickpeas, crushed chickpeas, wheat and casein were analyzed non-destructively for sulfur by the following procedure. Approximately 0.5 grams of sample were placed in a polyethylene vial which was placed in a polystyrene vial with approximately 0.25 grams of N.F. grade (sublimed) sulfur and 1-5 milligrams of calcium as Specpure $CaCO_3$ (Johnson, Mathey and Co.) This polystyrene vial was placed in the RSR of the KSU Triga Mark II reactor and irradiated for five minutes at 200-250 kW of power. The sample was counted immediately after irradiation, after a two minute transfer time, with a Ge(Li) semiconductor detector and the TMC 4096 multiparameter pulse-height analyzer set to optimize the photopeak resolution of calcium-49 and sulfur-37. The output in the region of the 3.1 MeV photopeaks was punched out on computer cards and the calculations were done by the IBM 360/50 computer at Kansas State University. A computer program was written to read the sulfur and calcium reference spectra and a complex cereal grain spectrum. The computer program "Shifts" shifted the complex spectrum to align the calcium peak in the reference and the complex spectra. The computer program "Unfold" unfolded the spectrum by a least-squares analysis.

A decay analysis was made on one run of whole chickpeas over a period of 18 hours to determine the level and half-life of the activity underlying the calcium-49 and sulfur-37 photopeaks. Another computer program extrapolated these "background" values back to the time the sample was counted for sulfur and calcium. These values were used as an estimate for "background".

A run was made on a standard dilute solution of ammonium sulfate to determine the lower limit of sensitivity of the neutron activation analysis for sulfur in an interference-free system. A 1.00 ml aqueous ammonium sulfate sample containing 0.3394 mg of sulfur was irradiated for thirty minutes in the RSR of the reactor along with a sulfur and calcium reference. The

irradiated samples were counted for ten minutes on the Ge(Li) analyzer system with the same analyzer settings used in the sulfur analysis of the cereal grains.

The nitrogen in the wheat, chickpeas, and casein was analyzed by the method of Eckhoff (17) and Wood (55). Approximately one gram of the sample was placed in a polyethylene vial. Nitrogen and phosphorus references were contained in separate vials and all were placed on the sample platform. This platform was rotated horizontally at 4 r.p.m. in front of the drift tube of the Texas-Nuclear 14.1 MeV neutron generator*. The platform was rotated to expose all samples to an equal neutron flux. The samples were irradiated for ten minutes and then counted for two minutes on the Ge(Li) semiconductor detector and analyzed with the TMC 4096 multiparameter pulse-height analyzer. The spectrum was not complex, so the unfolding and calculations were done by hand with the aid of a desk calculator.

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RESULTS AND DISCUSSION

The results of the nitrogen analyses are listed in Table I. The percent protein was obtained by the standard method, i.e., by multiplying the percent nitrogen by 6.25. This constant represents the average protein molecular weight divided by the weight of nitrogen in the protein.

The nitrogen-13 produced in the $^{14}N(n,2n)^{13}N$ reaction decays by positron emission. This decay is detected by the 0.511 MeV gamma from the annihilation of the positron. Phosphorus-30 from the $^{31}P(n,2n)^{30}P$ reaction also produces a 0.511 MeV gamma from the annihilation of a positron. The 1.78 MeV gamma of aluminum-28 from the $^{31}P(n,\alpha)^{28}Al$ reaction can be used to determine the contribution of ^{30}P to the 0.511 MeV photopeak. To account for the differences in the half-lives of ^{30}P and ^{28}Al, a calibration curve was made from a phosphorus reference to determine, at various decay times, the ratio of the 1.78 MeV photopeak to the 0.511 MeV photopeak. The curve was used to subtract the contribution of phosphorus-30 to the 0.511 MeV photopeak.

The average activity per channel from the five channels on either side of the photopeak was used as the background activity under the photopeak and subtracted from each channel. After the two subtractions, the 0.511 MeV photopeak activity was corrected for decay and compared with a nitrogen reference activity to determine the weight of nitrogen present.

The protein values from this analysis are not in close agreement with the protein values by Kjeldahl analysis. These Kjeldahl analyses were run by the Department of Grain Science, Kansas State University. In contrast, Wood (55) reported very good agreement between NAA and Kjeldahl protein analyses on various agricultural samples.

The data in Table II were taken from work done by Eckhoff and Ervin (18).

There is evidently very close agreement between NAA protein analysis of soybeans and the Kjeldahl protein analysis on the same samples run at the U.S. Regional Soybean Laboratory, Peoria, Illinois. These results suggest that these Kjeldahl protein analyses performed at KSU are in error.

The amount of sulfur in the various samples is listed in Table III. The analysis of these data was much more difficult and is discussed in several parts.

The area of interest of a typical ^{49}Ca-^{37}S complex spectrum of a grain sample is shown in Figure 1. Forty-one channels were chosen from each data set to perform the analysis. The channels were chosen so the ^{49}Ca photopeak was about the fifteenth channel. This group of channels included the entire reference ^{49}Ca and ^{37}S photopeaks, and about five channels beyond (higher energy) the ^{37}S photopeak. The extra channels were used to determine the "background" under each photopeak. These 41 channels were divided into three groups. The first 21 channels were chosen as the ^{49}Ca photopeak area. The next 15 channels were taken as the ^{37}S photopeak area. The last five channels were called the post-sulfur area and were used in the "background" estimation.

A whole chickpea sample was counted for 10 minute intervals over a period of about 18 hours and the resultant spectrum was treated as described above. A ratio was then established between the "background" activity in the post-sulfur area and the extrapolated "background" in the ^{49}Ca area and the ^{37}S area. A sample calculation is listed in Appendix I. This ratio was used to estimate the "background" under the ^{49}Ca and ^{37}S photopeaks in all the other complex spectra.

The "background" is the result of the summation of two separate gamma rays which enter the detector at the same time. The detector stores the pulse in a channel corresponding to the sum of the two energies. In this study,

manganese-56 and chlorine-38 caused the greatest amount of summing activity. One partial solution to this problem would be an electronic system that sorted incoming pulses faster. The result would be less pulse pile-up and less summing of energy of separate gamma rays.

Use of the same ratio for all runs corresponds to an assumption that all samples have the same amount of these interfering elements. This assumption is not necessarily true for wheat and certainly is not true for the casein and ammonium sulfate samples. Yet, the background activities of these two samples was so low (1-3 counts/channel) that applying the ratio did not alter the values significantly.

The "background" analysis was, without a doubt, only an estimate and probably represents the greatest source of error in the sulfur analysis. No background was substracted from the reference spectra. The error introduced by the neglect of this background was less than 3%.

The computer program used to fit and unfold the ^{49}Ca and ^{37}S photopeaks is listed in Appendix II. The program fits the complex data to the calcium reference data by the alignment of the two ^{49}Ca photopeaks. It was assumed that the slope (MeV/channel) remained constant between the reference spectrum and the complex spectrum, and that the difference in the photopeak channel was due to a base line shift. It was also assumed that there was no shift between the ^{49}Ca reference spectrum and the ^{37}S reference spectrum in any given run.

The computer program in subroutine "Gauss" establishes the channel number of the ^{49}Ca photopeak in the calcium reference and the complex spectra. The subroutine "Shifts" then shifts the complex spectrum, channel by channel. Subroutine "Unfold" is based on a weighted matrix which satisfies the Gauss-Markoff Theorem conditions to obtain minimum variance unbiased estimates of the amounts of calcium and sulfur present. This technique for fitting and

unfolding has been discussed by Eckhoff and Ervin (18). The subroutine "Unfold" also generates the standard error of the quantity of calcium and sulfur. It establishes a synthetic complex spectrum and compares it with the original to determine Chi-squared divided by the degrees of freedom, the quality of fit. The expected value of the quality of fit is unity. Table IV shows the effect on the quality of fit value of altering the number of channels used to establish the photopeak and to fit the data. The values reported in Table III are generally those with the lowest quality of fit value.

Since the casein and the ammonium sulfate sample contained less Ca than S, the ^{37}S photopeak and the sulfur reference were used to fit and unfold the complex spectra.

The preciseness of the NAA method for sulfur is shown by the closeness of agreement between the values for the ammonium sulfate obtained from this analysis and the theoretical value. The ammonium sulfate sample also gives an indication of the lower limit of this technique for sulfur analysis. After a 30 minute activation, the ^{37}S activity was essentially saturated. This resulted in a total photopeak activity of about 40 counts above the background. The background level for an interference-free sample, such as ammonium sulfate, was about 35 counts. There would be little benefit gained from the analysis of samples of lower sulfur concentrations by this method with the use of the present equipment. Therefore, a lower limit of about 300 μg was estimated for the equipment used in this study.

Dams et al., (12) reported a detection limit of 25 μg for analysis by NAA. They were apparently using an electronic system with a faster pulse sorting system. They reported a resolution of 2.5 KeV full width at half maximum for the 1332 KeV ^{60}Co photopeak. Perhaps they were also using a higher bias voltage on their Ge(Li) detector which would yield a greater counting efficiency

for the 3.1 MeV gamma rays. In addition, the thermal to fast neutron flux ratio of their reactor was different than the ratio available for this work. All of these factors influence the detection limit of an analysis and could explain the difference in the reported detection limit.

Calcium, manganese, and chlorine generate the most severe interference with this method of analysis. Any one of these three could be tolerated in rather large amounts. The problem arises when there is a combination of them in the sample, as in cereal grain samples.

A sample size of about 0.5 grams was used throughout this study. It was found that a larger sample size increased the interference from ^{56}Mn and ^{38}Cl to an intolerable level. Smaller sized samples reduced the sulfur activity to an undetectable level.

The chickpea and casein samples were run on an amino acid analyzer (15). The values for sulfur from the methionine and cystine are listed in Table V. The value for sulfur in chickpeas #391 by the NAA method is quite consistent with the value obtained by amino acid analysis. The "background" decay study was made on this sample and suggests that, with proper "background" subtraction, the NAA method is quite good.

The values for sulfur in the wheat and casein samples were likewise quite consistent with the expected value. The ratios of interfering ^{56}Mn, ^{38}Cl, and ^{49}Ca were more favorable for the resolution of the ^{37}S photopeak in the wheat and casein samples. Subsequently, the values for sulfur were close to the expected value.

In the chickpea #392 sample, the interference was severe and no "background" decay study was made. It appears that too low a value for the "background" was subtracted, with high values for sulfur as a result.

The S:N ratios for the samples studied are listed in Table VI.

The method described for the determination of the S:N ratio in cereal grains offers, upon further refinement, a fast, non-destructive method for estimating protein quality in cereal grains.

SUGGESTIONS FOR FUTURE RESEARCH

Application of the analytical techniques used in this work for the determination of the S:N ratio in soils and plant tissue would be of interest. Use of the sulfur analytical technique for the analysis of sulfur in soil extract solutions would also be of value in agricultural application.

Determination of the mechanism and products of the $HF \cdot HNO_3$ concentrated acid oxidation of cereal grains would be a worthwhile effort. This would include analysis of the insoluble products when such a solution is neutralized. The discovery of what happens to the calcium in such a solution would be of considerable interest.

A sulfur analysis in cereal grains by neutron activation from a Cf-252 source holds the greatest promise of an interference-free technique. The neutron energy distribution from a Cf-252 source has an average value of approximately 2.0 MeV and a significant neutron flux at 4 MeV which is the threshold energy of the $^{34}S(n,p)^{34}P$ reaction.

From this, one would predict that the $^{34}S(n,p)^{34}P$ reaction would occur while the interfering $^{16}O(n,p)^{16}N$ reaction would not occur. Calculation of the threshold energy for the $^{16}O(n,p)^{16}N$ reaction shows that neutron energies greater than 10 MeV are required to initiate the reaction.

The Cf-252 source also could be used for prompt gamma work.

Some correlations should be made between the standard Kjeldahl method and NAA for nitrogen in wheat. This would establish the validity of the nitrogen values for wheat listed in this work.

The analysis of the "background" must be modified and refined to do a more accurate job of estimating the amount to be subtracted from the various spectra.

Beta spectroscopy was not used in this study due to insufficient equipment. A study should be made to determine the merits of beta spectroscopy in sulfur analysis. It may be possible to detect ^{34}P in statistically significant quantities based on the beta decay of ^{34}P.

TABLE I

A summary of the nitrogen analyses on the samples used in this study.

Sample	% Protein by NAA	% Protein by Kjeldahl analysis
Chickpeas #392	27.70 ± 0.61	19.8 (a)
Chickpeas #391	26.22 ± 3.92	18.2 (a)
Wheat	16.57 ± 1.19	14.8 (b)
Casein	104.5 ± 7.4	80-90 (a)

(a) DeYoe, C. Department of Grain Science, KSU

(b) Schrenk, W.G. Kansas State U. Ag. Expt. Sta. Tech. Bull. 79 (1955)

TABLE II

Comparison of NAA and Kjeldahl protein analyses on several soybean samples from U.S. Regional Soybean Laboratory.

% Protein by NAA	% Protein by Kjeldahl
39.1 ± 3.9	38.9
39.6 ± 4.0	40.3
35.7 ± 3.6	39.1
41.7 ± 4.2	39.2
40.1 ± 4.0	40.1
37.3 ± 3.7	38.9
36.9 ± 3.7	40.4
43.3 ± 4.3	41.3
35.9 ± 3.6	39.3
35.4 ± 3.5	40.6
37.8 ± 3.8	40.9

TABLE III

A summary of the sulfur analyses on the samples used in this study.

Run. No.		Sulfur(ppm)	Quality of Fit
34	Chickpeas #392 (whole)	3119. ± 354.	12,806
37	Chickpeas #392 (whole)	4011. ± 317.	14,486
35	Chickpeas #392 (crushed)	4217. ± 352.	7,736
38	Chickpeas #392 (crushed)	3027. ± 305.	16.366
36	Wheat	1508. ± 202.	2,586
39	Wheat	720. ± 146.	3,088
40	Chickpeas #391 (whole)	1390. ± 260.	9,603
41	Casein	5835. ± 355.	2,907
43	Casein	6685. ± 303.	2,769
46	$(NH_4)_2SO_4$	321. ± 49.	2,026
	Theoretical value	339. ± 0.2	

TABLE IV

The effect of altering the number of channels used to establish the photopeak and fit the data on the quality of fit.

Channel Nos. used calcium reference spectrum	Channel Nos. used in complex spectrum	Quality of fit
	Run #38 Chickpeas #392 (ground)	
8-22	10-22	13.9897
14-19	14-19	16.366
15-18	15-18	24.4735
	Run #41 Casein	
22-33	26-34	3.5934
25-32	26-32	1.9625
28-31	28-31	2.9074

TABLE V

A comparison of the quantity of sulfur found in the samples under study by NAA and by total amino acid analysis.

Sample	Sulfur by NAA (ppm)	Sulfur by amino acid analysis (ppm)
Chickpeas #392	3593.*	1620.
Chickpeas #391	1390.	1551.
Wheat	1114.	1200.-2000. (a)
Casein	6086.	5390.

* See text

(a) Schrenk, W.G. Kansas State U. Ag. Expt. Sta. Tech. Bull. 136 (1964)

TABLE VI

The S:N ratio by NAA of the samples under study.

Sample	S:N Ratio
Chickpeas #392	0.812
Chickpeas #391	0.441
Wheat	0.421
Casein	0.0363

FIGURE 1

The area of interest of a typical complex cereal grain spectrum.

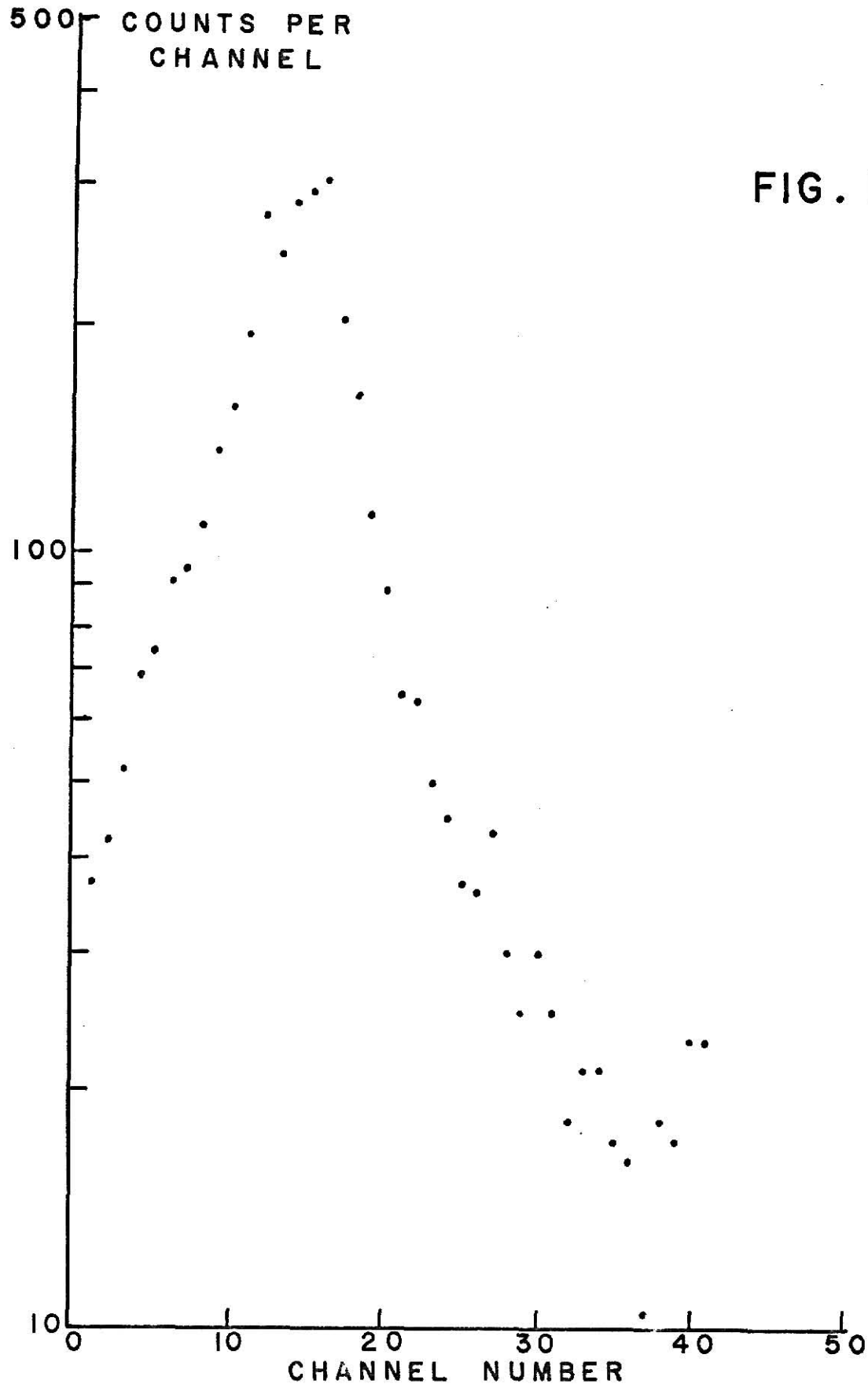


FIG. 1

Appendix I

A sample calculation of the "background".

Data from Run #40: Whole Chickpeas #392

Decay time(t) (min.)	^{49}Ca area (21 channels)	^{37}S area (15 channels)	Post ^{37}S area (5 channels)
130	2.24 c/ch.	1.4 c/ch.	1.6 c/ch.
2. (Sample count time extrapolated by computer.)	24.5 c/ch.	17.2 c/ch.	

$$\text{Ratio I} = \frac{^{49}\text{Ca area @ t = 2.m}}{^{49}\text{Ca area @ t = 130.m}} = \frac{24.5}{2.24} = 11.4$$

$$\text{Ratio II} = \frac{^{37}\text{S area @ t = 2.m}}{^{49}\text{S area @ t = 130.m}} = \frac{17.2}{1.4} = 12.3$$

(Ratio I) x (Post ^{37}S area @ t = 130.m) = 17.5 c/ch.

(Ratio II) x (Post ^{37}S area @ t = 130.m) = 19.65 c/ch.

Average = 18.6 c/ch. = Post ^{37}S area @ t = 130.m

$$\text{Ratio for } ^{49}\text{Ca area} = \frac{24.5}{18.6} = 1.32$$

$$\text{Ratio for } ^{37}\text{S area} = \frac{17.2}{18.6} = 0.925$$

Applying this to:

Run #34-- Whole chickpeas #391

Post ^{37}S area = 8.4 counts/channel

"Background" for ^{49}Ca area = (1.32)(8.4) = 11.0 counts/channel

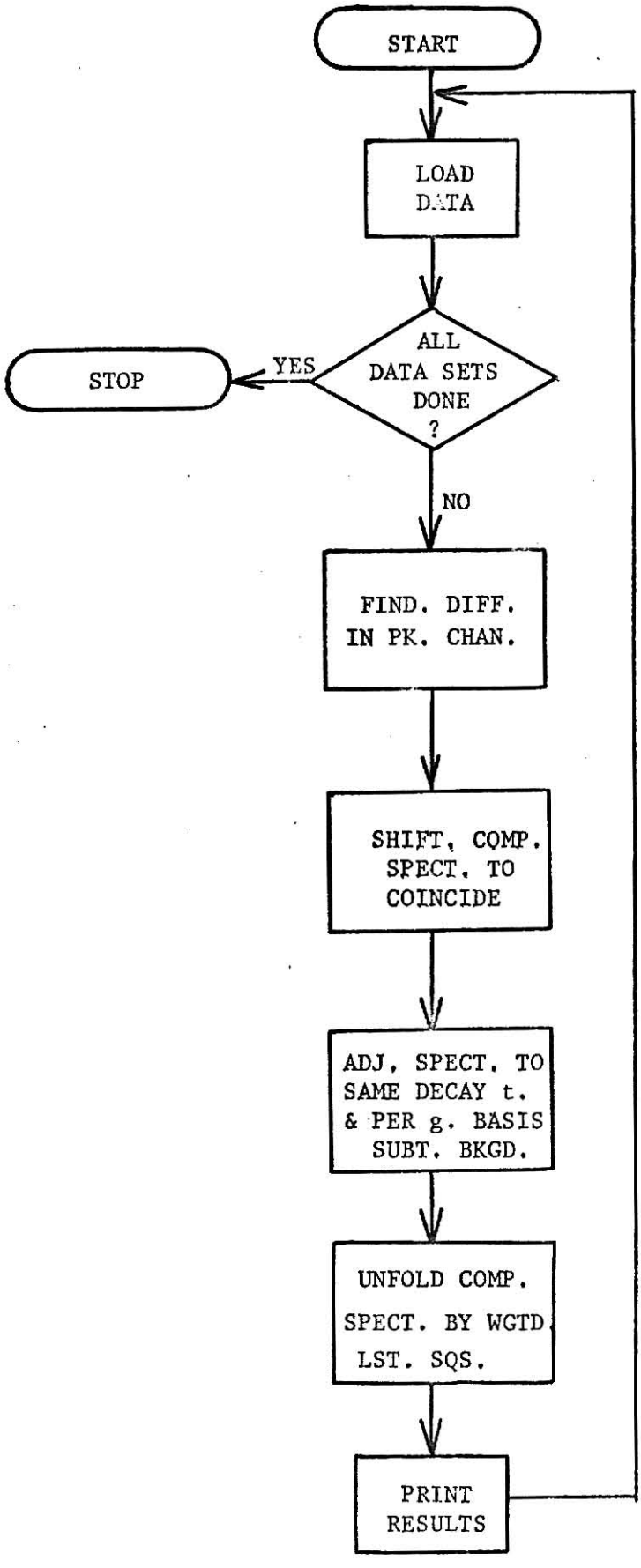
"Background" for ^{37}S area = (0.925)(8.4) = 7.7 counts/channel

Run #41-- Casein

Post ^{37}S area = 2.0 counts/channel

"Background" for ^{49}Ca area = (1.32)(2.0) = 2.7 counts/channel

"Background" for ^{37}S area = (0.925)(2.0) = 1.89 counts/channel



Appendix II

The flow diagram and computer programs used in the sulfur data analysis,

MAIN PROGRAM

```
      IMPLICIT REAL*8(A-H,O-Z)
      DIMENSION SPECT(99),O(20),CA(99),SUL(99),ACA(20),ASUL(20),
     1VAR(99),C(4),SCP(99)
    9 FORMAT(///' BKGD AREA FROM CAL= ',F6.2,3X,' BKGD AREA FROM SUL= ',
     1F6.2)
   10 FORMAT(8F10.2)
   11 FORMAT(20A4)
   12 FORMAT(6X,12F6.0,2X)
   13 FORMAT(///' NON-SHIFTED COMPLEX SPECTRUM')
   14 FORMAT(3I3)
   15 FORMAT(//' SHIFTED COMPLEX SPECTRUM')
   17 FORMAT(1H1,'NON-SHIFTED CA SPECTRUM')
   18 FORMAT(///' NON-SHIFTED SUL SPECTRUM')
   19 FORMAT(3I3,5F10.4)
   20 FORMAT(6X,2F6.2)
  100 READ(1,14)MODE
      IF(MODE.GT.0)CALLEXIT
      DO 180 LOOP=1,3
      READ(1,14)       NCHNCP,IPPLL,IPPUL
      IF(LOOP.GT.1)GO TO 150
      READ(1,11)O
      READ(1,19)NO,NCHAN1,NCHAN2,CALDT,SULDT,CALW,SULW,SAMW
      READ(1,20)BKG1,BKG2
      READ(1,12)(SPECT(I),I=1,48)
  150 DO 132 J=IPPLL,IPPUL
  132 VAR(J)=SPECT(J)
      CALL GAUSS(SPECT,NCHNCP,IPPLL,IPPUL,BO,YO,XOCP,C,VAR)
      READ(1,14)       NCHNCA,IPPLL,IPPUL
      IF(LOOP.GT.1)GO TO 160
      READ(1,11)ACA
      READ(1,12)(CA(I),I=1,48)
  160 DO 133 J=IPPLL,IPPUL
  133 VAR(J)=CA(J)
      CALL GAUSS(CA,NCHNCA,IPPLL,IPPUL,BO,YO,XOCA,C,VAR)
      BLSHFT=XOCP-XOCA
      IF(LOOP.GT.1)GO TO 170
      READ(1,11)ASUL
      READ(1,12)(SUL(I),I=1,48)
  170 CALL SHIFTS(SPECT,SCP,1.0,48,48,BLSHFT)
      IF(LOOP.GT.1)GO TO 171
      WRITE(3,19)NO,NCHAN1,NCHAN2,CALDT,SULDT,CALW,SULW,SAMW
      WRITE(3,17)
      WRITE(3,11)ACA
      WRITE(3,12)(CA(I),I=1,48)
      WRITE(3,11)O
      WRITE(3,13)
      WRITE(3,12)(SPECT(I),I=1,48)
  171 WRITE(3,15)
      WRITE(3,11)O
      WRITE(3,10)BLSHFT
      WRITE(3,12)(SCP(I),I=1,48)
      IF(LOOP.GT.1)GO TO 175
      WRITE(3,18)
      WRITE(3,11)ASUL
      WRITE(3,12)(SUL(I),I=1,48)
      WRITE(3, 9)BKG1,BKG2
  175 WRITE(3,14)IPPLL,IPPUL
      CALL UNFOLD(NO,NCHAN1,NCHAN2,CALDT,SULDT,CALW,SULW,SAMW, CA, SUL,
     1SCP,BKG1,BKG2,LOOP)
  180 CONTINUE
      GO TO 100
      END
```

SUBROUTINE GAUSS

```
      SUBROUTINE GAUSS(SPECT,NCHN,IPPLL,IPPUL,BO,YO,XO,C,VAR)
      IMPLICIT REAL*8(A-H,O-Z)
      DOUBLE PRECISION DABS,DSQRT,DEXP,DLOG,DFLOAT
      DIMENSION SPECT(48),A(3,3),B(3),C(4),B2(30),VAR(48)
    4 FORMAT(1HO,4X,'LOWER LIMIT OF ALPHAPEAK FROM CHANNEL',I4,' TO CHAN
     1NEL',I4,10X,'UPPER LIMIT OF ALPHAPEAK FROM CHANNEL',I4,' TO CHANNE
     2L',I4)
    5 FORMAT(1HO,33X,'EXPERIMENTAL ALPHAPEAK POINTS',10X,'CALCULATED ALP
     1HAPEAK POINTS')
    6 FORMAT(    45X,F6.0,33X,F6.0)
   14 FORMAT(1HO,33X,' FUNCTION ERROR = ',E14.8,10X,'LN ERROR = ',E14.8)
      F(X)=YO*DEXP(-((X-XO)**2)/BO)
      IPPLO=IPPLL
      IPPUO=IPPUL
  212 XN=0.
      DO 20 I=1,3
      B(I)=0.
      DO 20 J=1,3
   20 A(I,J)=0.
C**** FORM SOLUTION MATRIX
      DO 30 I=IPPLL,IPPUL
      XN2=XN*XN
      XN3=XN2*XN
      XN4=XN3*XN
      ZN=DLOG(SPECT(I))
      A(1,1)=A(1,1)+XN4*VAR(I)
      A(1,2)=A(1,2)+XN3*VAR(I)
      A(1,3)=A(1,3)+XN2*VAR(I)
      A(2,3)=A(2,3)+XN*VAR(I)
      A(3,3)=A(3,3)+VAR(I)
      B(1)=B(1)+XN2*ZN*VAR(I)
      B(2)=B(2)+XN*ZN*VAR(I)
      B(3)=B(3)+ZN*VAR(I)
   30 XN=XN+1.
      A(2,1)=A(1,2)
      A(3,1)=A(1,3)
      A(2,2)=A(1,3)
      A(3,2)=A(2,3)
      CALL INVERT(3,A,B)
      XO=(-B(2)/(2.*B(1)))
      BO=-1./B(1)
      Y1=0.
      Y2=0.
      XN=0.
      DO 104 I=IPPLL,IPPUL
      Y1=Y1+DEXP(-(XN-XO)**2/BO)
      Y2=Y2+DEXP(-2*(XN-XO)**2/BO)/SPECT(I)
  104 XN=XN+1.0
      YO=Y1/Y2
      C(3)=1./Y2
      XO=XO+DFLOAT(IPPLL)
      N=0
      RES=0.
      STDEV=0.
      XN=0.0
      DO 40 I=IPPLL,IPPUL
      STDEV=STDEV+((DLOG(SPECT(I))-B(3)-B(2)*XN-B(1)*XN**2)**2)
      RES=RES+(SPECT(I)-F(DFLOAT(I)))**2
   40 XN=XN+1.
      STDEV=DSQRT(RES/DFLOAT(IPPUL-IPPLL-2))
      ADEV=SPECT(IPPLL)-F(DFLOAT(IPPLL))
      ADEV2=SPECT(IPPUL)-F(DFLOAT(IPPUL))
      TST2=2.0*STDEV
 2000 FORMAT(3E15.8)
      WRITE(3,2000)TST2,ADEV,ADEV2
      IF(2.0*STDEV.GT.DABS(SPECT(IPPLL)-F(DFLOAT(IPPLL))))GO TO 50
      IPPLL=IPPLL+1
      N=1
   50 IF(2.*STDEV.GT.DABS(SPECT(IPPUL)-F(DFLOAT(IPPUL))))GO TO 60
      IPPUL=IPPUL-1
      N=1
   60 IF(N.EQ.1) GO TO 212
      IF(IPPLO.NE.IPPLL) GO TO 70
      IF(IPPUO.EQ.IPPUL)GO TO 71
   70 CONTINUE
   71 ZN=1./(4.*B(1)**2)
      XN=(B(2)/B(1))**2
      XN2=1./(B(1)**4)
      XN3=XN/4.
      XN4=(XN**2)/DFLOAT(NCHN)
      C(1)=ZN*(A(2,2)+XN*A(1,1))
      C(2)=XN2*A(1,1)
      C(4)=0.6922*C(2)/BO
      DO 100 I=1,4
  100 C(I)=DSQRT(    C(I))
      MAX=IPPUL-IPPLL+1
      XN=0.
      XO=XO-DFLOAT(IPPLL)
      DO 105 I=1,MAX
      B2(I)=F(XN)
  105 XN=XN+1.
      XO=XO+DFLOAT(IPPLL)
      RETURN
      END
```

SUBROUTINE INVERT

```
      SUBROUTINE INVERT(NISO,R,B)
      IMPLICIT REAL*8(A-H,O-Z)
      DOUBLE PRECISION DABS,DSQRT,DEXP,DLOG
      DIMENSION R(3,3),RI(3,3),C( 6),B(3)
C
C        INVERT RESPONSE MATRIX BY BORDERING METHOD
C
      R(1,1)=1./R(1,1)
      ISO = 1
  120 ISO=ISO+1
      MAX=ISO-1
      ANN=0.
      DO 110 J=1,NISO
      DO 110 I=1,NISO
  110 RI(I,J)=0.
      DO 101 I=1,MAX
  101 C(I)=R(I,ISO)
      DO 102 J=1,MAX
      DO 102 I=1,MAX
  102 ANN=ANN+  C(I)*R(I,J)*C(J)
      RI(ISO,ISO)=1./(R(ISO,ISO)-ANN)
      DO 103 J=1,MAX
      DO 103 I=1,MAX
      RI(J,ISO)=RI(J,ISO)-R(J,I)*  C(I)*RI(ISO,ISO)
      RI(ISO,J)=RI(J,ISO)
      DO 103 K=1,MAX
      DO 103 L=1,MAX
  103 RI(I,J)=RI(I,J)+R(I,L)*  C(L)*  C(K)*R(K,J)
      DO 104 I=1,MAX
      DO 104 J=1,MAX
  104 RI(I,J)=RI(I,J)*RI(ISO,ISO)+R(I,J)
      DO 105 I=1,ISO
      DO 105 J=1,ISO
  105 R(I,J)=RI(I,J)
      IF(ISO.LT.NISO) GO TO 120
      DO 106 J=1,NISO
    3 FORMAT(5E15.8)
      C(J)=0.0
      DO 106 I=1,NISO
  106 C(J)=C(J)+R(J,I)*B(I)
      DO 107 I=1,NISO
  107 B(I)=C(I)
      RETURN
      END
```

SUBROUTINE SHIFTS

```
      SUBROUTINE SHIFTS(SPECT,S,RATIO,NCHN,NCHN2,BLSHFT)
      IMPLICIT REAL*8(A-H,O-Z)
      DIMENSION SPECT(48),S(48)
      X=0.
      II=0
      NR=0
   22 X=X+1.
      II=II+1
      XP=(X/RATIO)+BLSHFT
      IF(XP.LE.1.)GO TO 24
      IF(XP.GE.2.)GO TO 25
   24 S(II)=(SPECT(1)+(SPECT(1)-SPECT(2))*(1.0-XP))/RATIO
      GO TO 22
   25 IX2=XP+1.0
      IX1=IX2-1
      IF(IX2.GT.48) GO TO 4000
      Y1=SPECT(IX1)
      Y2=SPECT(IX2)
      GO TO 28
 4000 IX1=47
      IX2=48
      Y1=SPECT(48)
      Y2=SPECT(48)
      NR=1
   28 S(II)=(Y1+(XP-IX1)*(Y2-Y1))/RATIO
      IF(S(II).LT.0.)S(II)=0.0
      IF(NR.GT.0) RETURN
      IF(II.LT.NCHN2)GO TO 22
      RETURN
      END
```

SUBROUTINE UNFOLD

```
      SUBROUTINE UNFOLD(NO,NCHAN1,NCHAN2,CALDT,SULDT,CALW,SULW,SAMW,X1,
     1X2,Y,BKG1,BKG2,LOOP)
      IMPLICIT REAL*8(A-H,O-Z)
      DIMENSIONX1( 99),X2( 99),Y( 99),YC( 99),DIFF( 99),BC( 99),BS( 99)
   21 FORMAT(' CAL = ',E14.7,3X,'  SDCAL = ',E14.7)
   22 FORMAT(' SUL = ',E14.7,3X,'  SDSUL = ',E14.7)
   23 FORMAT(1X,'CHI SQD BY DF=',E14.7)
   24 FORMAT(4X,'ORIG',8X,'CAL',8X,'SUL',7X,'CALC',7X'DIFF')
   25 FORMAT(5(1X,F10.2))
      NO1=NO-6
      IF(LOOP.GT.1) GO TO 90
      F1=DEXP(0.693*CALDT/8.8)/CALW
      F2=DEXP(0.693*SULDT/5.1)/SULW
      DO 90 I=1,NO1
      X1(I)=X1(I)*F1
      X2(I)=X2(I)*F2
   90 CONTINUE
      DO99I=1,NO1
      IF(I.GT.NCHAN1)GOTO105
      Y(I)=(Y(I)-BKG1)/SAMW
      GO TO 102
  105 Y(I)=(Y(I)-BKG2)/SAMW
  102 IF(Y(I).LE.0.0)Y(I)=1.0
   99 CONTINUE
      X1SQDY=0.
      X2SQDY=0.
      X1X2DY=0.
      X1S=0.
      X2S=0.
      DO100I=1,NO1
      X1SQDY=X1SQDY+X1(I)**2/Y(I)
      X2SQDY=X2SQDY+X2(I)**2/Y(I)
      X1X2DY=X1X2DY+(X1(I)*X2(I))/Y(I)
      X1S=X1S+X1(I)
  100 X2S=X2S+X2(I)
      DW=X1SQDY*X2SQDY-(X1X2DY)**2
      BCAL=(X2SQDY*X1S-X1X2DY*X2S)/DW
      SSQB1=X2SQDY/DW
      SDB1=DSQRT(SSQB1)
      BSUL=(-X1X2DY*X1S+X1SQDY*X2S)/DW
      SSQB2=X1SQDY/DW
      SDB2=DSQRT(SSQB2)
      CHI=0.
      DO101I=1,NO1
      BC(I)=BCAL*X1(I)
      BS(I)=BSUL*X2(I)
      YC(I)=BC(I)+BS(I)
      DIFF(I)=Y(I)-YC(I)
  101 CHI=CHI+DIFF(I)**2/YC(I)
      CHI=CHI/(DFLOAT(NO1)-2.0)
      WRITE(3,21)BCAL,SDB1
      WRITE(3,22)BSUL,SDB2
      WRITE(3,23)CHI
      WRITE(3,24)
      WRITE(3,25)(Y(I),BC(I),BS(I),YC(I),DIFF(I),I=1,NO1)
      RETURN
      END
```

BIBLIOGRAPHY

1. Agazzi, E.J., Parks, T.D., and Brooks, F.R. Anal. Chem. 23:1011 (1951)
2. Alexander, G.V. Anal. Chem. 37:1671 (1965)
3. Allaway, W.H. and Thompson, J.F. Soil Sci. 101:230 (1966)
4. Association of Official Agricultural Chemists. Official and Tentative Methods of Analysis. Fifth edition (1940)
5. Axelrod, H.D., Bonelli, J.E., and Lodge, J.P. Anal. Chem. 42:512 (1970)
6. Bardsley, C.E.,Jr. and Jordan, H.V. Agron. J. 49:310 (1957)
7. Bertolacini, R.J. and Barney, J.E.II Anal. Chem. 29:281 (1957)
8. Bird, P.R. and Fountain, R.D. Analyst 95:98 (1970)
9. Bloomfield, C. Analyst 87:586 (1962)
10. Brown, G. and Kanaris-Sotiriou, R. Analyst 94:782 (1969)
11. Butters, B. and Chenery, E.M. Analyst 84:239 (1959)
12. Dams, R., Robbins, J.A., Rahn, K.A. and Winchester, J.W. Anal. Chem. 42:861 (1970)
13. Dean, G.A. Analyst 91:530 (1966)
14. Debrun, J.L. and Albert, P. Bull. Soc. Chim. Fr. 3:1017 (1969)
15. DeYoe, C. Private Communications (April 1970)
16. Dixon, J.P. Talanta 4:221 (1960)
17. Eckhoff, N.D. Unpublished Data (1970)
18. Eckhoff, N.D. and Ervin, P. In publication preparation (1970)
19. Elejalde, C. and Albisu, F. Easayos Invest. 3:48 (1968)
20. Ervin, P. M.S. Thesis (unpublished) (1970)
21. Evans, C.A., and Rost, C.O. Soil Science 59:7 (1945)
22. Frear, D.E. J. Biol. Chem. 86:285 (1930)
23. Fujii, I., Inouye, T., Muto, H., Onodera, K., and Tani, A. Analyst 94:189 (1969)
24. Gersonde, K. Anal. Biochem. 25:459 (1968)

25. Gillespie, E. Power III:69 (1967)

26. Goldman, D.T. and Roesser, J.R. Chart of the Nuclides. G.E. Co. Ninth edition (1966)

27. Gustafsson, L. Talanta 4:227 (1960)

28. Hughes, J. and Harvey, J.A. Neutron Cross Sections. Brookhaven National Laboratory (1955)

29. Iismaa, O. J. Aust. Inst. Agr. Sci. 125:136 (1959)

30. Jenkinson, D.S. Analyst 93:535 (1968)

31. Johnson, C.M. and Nishita, H. Anal. Chem. 24:736 (1952)

32. Johnson, C.M. and Ulrich, A. Calif. Ag. Expt. Sta. Bull. 766:25 (1959)

33. Martin, J.M. and Stephen, W.I. Anal. Chim. Acta. 39:175 (1967)

34. Massoumi, A. and Cornfield, A.H. Analyst 88:321 (1963)

35. McCandless, E.L. Anal. Biochem. 7:357 (1964)

36. Mendes-Bezerra, A.E. and Uden, P.C. Analyst 94:308 (1969)

37. Miller, D.S. and Donoso, G. J. Sci. Fd. Agric. 4:345 (1963)

38. Miller, D.S. and Naismith, D.J. Nature 182:1786 (1958)

39. Morrison, F.B. Feeds and Feeding. The Morrison Publishing Co. Clinton, Iowa. 22nd edition

40. Niese, S. Kernenergie 8:499 (1965)

41. Pellett, P.L., Kantarjian, A., and Jamalian, J. J. Sci. Fd. Agric. 20:229 (1969)

42. Pippen, E.L. and Mecchi, E.P. J. Agric. Fd. Chem. 18:301 (1970)

43. Rafsalski, H., Ponomarenko, W., and Nogal, E. Rocz. Panstw. Zakl. Hig. 19:619 (1968)

44. Ross, W.J. Div. Tech. Inf. Index TID-7015. Oak Ridge National Laboratory Master Analytical Manual (1953-1964) (1960)

45. Schrenk, W.G. Kansas State U. Ag. Expt. Sta. Tech. Bull. 79 (1955)

46. Schrenk, W.G. Kansas State U. Ag. Expt. Sta. Tech. Bull. 136 (1964)

47. Shaw, W.M. J. Agr. Fd. Chem. 9:18 (1961)

48. Siegfriedt, R.K., Wiberley, J.S., and Moore, R.W. Anal. Chem. 23:1008 (1951)

49. Soep, H. and Demoen, P. Microchem. J. 4:77 (1960)

50. Spencer, K. Aust. J. Agr. Res. 10:500 (1959)

51. Steinbergs, A. Analyst 80:457 (1955)

52. Steinbergs, A., Iismaa, O., Freney, J.R. and Barrow, N.J. Anal. Chim. Acta 27:158 (1962)

53. Stewart, B.A. and Porter, L.K. Agron. J. 61:267 (1969)

54. Tisdale, S.L., Davis, R.L., Kinsley, A.F., and Hertz, E.T. Agron. J. 42:221 (1950)

55. Wood, D.E. Kaman Nuclear Tech. Bull. KN-65-186 (August 1965)

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DETERMINATION OF THE SULFUR TO NITROGEN RATIO IN CEREAL GRAINS BY NEUTRON ACTIVATION ANALYSIS

by

JAMES EDWARD ASSINK

B.A., Western Washington State College, 1967

AN ABSTRACT OF A MASTER'S THESIS

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1970

ABSTRACT

A neutron activation analysis technique was developed to determine protein quality in cereal grains by the sulfur to nitrogen ratio. The nitrogen analysis was based on the $^{14}N(n,2n)^{13}N$ reaction with 14.1 MeV neutrons.

Several different approaches were used in the sulfur analysis. Attempts were made to analyze sulfur by the fast neutron reaction $^{34}S(n,p)^{34}P$. The ^{34}P activity produced was too low to analyze after irradiation in a reactor flux. The ^{16}N produced from the $^{16}O(n,p)^{16}N$ reaction interfered with ^{34}P when the sample was irradiated with 14.1 MeV neutrons. An analysis based on the $^{32}S(n,p)^{32}P$ reaction was tried. Interference from the $^{31}P(n,\gamma)^{32}P$ reaction was severe and could not be reduced to tolerable levels by shielding the sample with cadmium.

An analysis based on the $^{36}S(n,\gamma)^{37}S$ reaction was tried. Calcium-49 interfered with the ^{37}S. Grain samples were oxidized by an $HF{\cdot}HNO_3$ concentrated acid mixture. The neutralized solution was passed through a cation exchange column to remove calcium. Calcium was not removed from this solution.

The analysis finally used involved counting the sample with a Ge(Li) semiconductor detector connected to a TMC 4096 multiparameter pulse-height analyzer set to optimize the photopeak resolution between ^{37}S and ^{49}Ca.

The values obtained for interference-free samples agreed well with theoretical values. Manganese, chlorine, and calcium were found to be severe interferences in cereal grains. These made the estimation of background activity under the photopeaks a very difficult problem which was not completely resolved in this study.