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# SULFURIC ACID–SELENIUM DIGESTION FOR MULTI-ELEMENT ANALYSIS IN A SINGLE PLANT DIGEST

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## ABSTRACT

At the ICRISAT soil chemistry laboratory, a sulfuric acid–selenium (Se) digestion method has been used for several years for determination of nitrogen (N) and phosphorus (P) in a single plant digest. The method is simple and was evaluated for determining N, P, potassium (K), Calcium (Ca), and magnesium (Mg) in plant samples using a single digest. Finely ground plant materials of pigeonpea (*Cajanus cajan* L.) and rice (*Oryza sativa* L.) plant samples were digested at 370°C. Digestion completed in about 2.5 h when the digests were clear and colorless. Results with plant samples having a wide range in elemental concentrations, showed that there was an excellent agreement in the values of various elements determined by the proposed digestion procedure and the routinely used Kjeldahl and triacid acid

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digestion methods. These results suggest that sulfuric acid–Se mixture can be used for preparing plant digests for determination of N, P, K, Ca, and Mg in a single digest. In addition, this method is adapted for preparing plant digests for automated determination of N and P in plant samples using a Technicon Autoanalyser II.

## INTRODUCTION

In preparing plant materials for elemental analysis, organic matter is destroyed using combustion at high temperature. This is done through dry ashing of plant materials using acid mixtures to digest the materials.[1,2]

In the past, attempts have been made to develop digestion procedures that can be used to determine several nutrient elements in a single plant digest. For example, the use of nitric and perchloric acid mixture[3,4] and sulfuric acid and hydrogen peroxide[2] have been proposed for determining macro- and micronutrient elements in a single plant digest. In our laboratory, we have been using an automated colorimetric procedure for the estimation of nitrogen (N), phosphorus (P) using a single digest.[5] This method uses sulfuric acid containing selenium (Se) to digest and prepare plant materials for N and P analysis in the same digest. At the ICRISAT (International Crops Research Institute for the Semi-Arid Tropics), this method of preparing digests, has been used for the past several years for routinely determining N and P using automated colorimetric methods[5] in diverse plant materials of sorghum (*Sorghum bicolor* L.), pearl millet (*Pennisetum glaucum* L.), groundnut (*Arachis hypogaea* L.), pigeonpea (*Cajanus cajan* L.), chickpea (*Cicer arietinum* L.), and maize (*Zea mays* L.) (Sahrawat et al., unpublished results).

Research reported here evaluated the possibility of using sulfuric acid–Se digestion mixture for preparing plant materials for manual determination of N, P, potassium (K), calcium (Ca), and magnesium (Mg) in the same digest using routine methods of analyses. The digestion procedure seems attractive in that it is simple and relatively safe, and requires fewer chemical reagents. Although it is realized that Se is a hazardous waste. But it should not pose a serious problem in the acidic soils of the humid zone that are not affected by salts. The use of digestion tubes with a block digester makes it possible to attain a stipulated temperature with precision for digesting plant materials. These considerations prompted exploring the use of sulfuric acid–Se digestion mixture for preparation of plant materials for multi-elemental analyses in a single digest.

## MATERIALS AND METHODS

### Plant Materials

Pigeonpea (*Cajanus cajan* L.) and rice (*Oryza sativa* L.) plant samples were used to compare results for determining N, P, K, Ca and Mg using the proposed digestion method with those obtained using the regular Kjeldahl digestion method for N and triacid digestion procedure for the estimation of K, Ca, and Mg.[6] Pigeonpea was chosen because its tissue woody in nature provides an ideal plant material for evaluating a digestion method. To provide additional evidence on the efficacy of the digestion technique, rice plant samples having a range in N, P, K, Ca and Mg were included. The plant materials were finely ground then oven dried at 60°C for 48 h before analysis by various procedures. The plant samples were selected from a number of a large number of samples to obtain a range in N, P, K, Ca, and Mg concentrations.

### Digestion Procedure

Approximately 0.5 g of finely ground plant samples were weighed and transferred to 250 mL digestion tubes. Fourteen [14] mL of concentrated sulfuric acid containing 0.5% Se (by wt as metal) powder was added to soak the plant material held in each tube. Sulfuric acid and Se mixture is prepared by dissolving Se powder in concentrated sulfuric acid by heating on a hot plate with occasional mixing by stirring with a glass rod. Five g of Se powder was added to about 500 mL of sulfuric acid and heated to dissolve the Se powder. The mixture was cooled and the volume made to one liter. After adding the digestion mixture to plant materials, the digestion tubes were transferred to block digester, preheated to 370°C temperature. About 2.5 h are needed for completing the digestion, indicated by clear and colorless plant digests.

To overcome an under-estimation of Ca by the proposed sulfuric acid–Se method because of formation of relatively insoluble calcium sulfate, plant digests were diluted with water (30 to 40 mL of distilled water) and left overnight. We have observed that about 16 h are needed for a complete solubilization of the calcium sulfate formed during digestion of plant materials.[7] This modification was also followed while analyzing plant materials for using the triacid digestion procedure. All plant analyses were carried out in duplicate and results reported are mean values of duplicate samples not differing significantly ($P = 0.05$).

The digests were made to 250 mL with distilled water. Suitable aliquots of digests were used to determine N by distillation with sodium hydroxide, P by the phosphovanadomolybdate colorimetric procedure, and K, Ca, and Mg using atomic absorption spectrophotometer.[6]

***Table 1.*** Comparison of Values of N Determined by the Sulfuric Acid–Se Digestion and Kjeldahl Digestion Procedures in Pigeonpea Plant Samples

| | N Concentration ($g\,100\,g^{-1}$) in Dry Plant Samples | |
|---|---|---|
| Sample No. | Sulfuric Acid–Se Digestion | Micro Kjeldahl Digestion |
| 1 | 3.75 | 3.89 |
| 2 | 3.88 | 3.89 |
| 3 | 4.74 | 4.62 |
| 4 | 3.70 | 3.64 |
| 5 | 3.87 | 3.71 |
| 6 | 3.75 | 3.51 |
| 7 | 2.43 | 2.32 |
| 8 | 2.33 | 2.29 |
| 9 | 2.12 | 2.17 |
| 10 | 1.07 | 1.05 |
| 11 | 1.13 | 1.06 |
| 12 | 1.62 | 1.49 |
| 13 | 1.11 | 1.14 |
| 14 | 1.05 | 1.03 |
| 15 | 1.01 | 1.03 |
| 16 | 0.75 | 0.72 |
| 17 | 0.67 | 0.59 |
| 18 | 0.59 | 0.62 |
| 19 | 0.81 | 0.86 |
| Mean | 2.13 | 2.09 |
| SD | 1.39 | 1.36 |

The difference between the values of N determined by the two digestion methods was not significant ($P = 0.05$). SD is standard deviation of mean.

The results of elemental analyses using the sulfuric acid–Se digestion procedures were compared with those obtained using routine digestion methods. Nitrogen was determined using the regular micro Kjeldahl digestion and distillation procedure. Phosphorus, K, Ca, and Mg were determined in plant digests prepared using triacid digestion mixture of nitric:sulfuric:perchloric acids in the ratio (v/v) of 9:2:1.[7] Plant materials (0.5 g) were digested in 125 mL conical flasks using 12 mL of triacid mixture. Phosphorus, K, Ca, and Mg in the plant digests were determined as described earlier.

***Table 2.*** Comparison of Values of P and K Determined by the Sulfuric Acid–Se Digestion and Triacid Digestion Procedures in Pigeonpea Plant Samples

| | P Concentration ($g\,100\,g^{-1}$) | | K Concentration ($g\,100\,g^{-1}$) | |
|---|---|---|---|---|
| Sample No. | Sulfuric Acid–Se | Triacid | Sulfuric Acid–Se | Triacid |
| 1 | 0.23 | 0.23 | 1.69 | 1.68 |
| 2 | 0.26 | 0.24 | 1.80 | 1.68 |
| 3 | 0.39 | 0.35 | 1.74 | 1.71 |
| 4 | 0.27 | 0.27 | 1.71 | 1.73 |
| 5 | 0.26 | 0.27 | 1.79 | 1.70 |
| 6 | 0.25 | 0.25 | 1.82 | 1.72 |
| 7 | 0.20 | 0.18 | 0.95 | 0.98 |
| 8 | 0.17 | 0.17 | 0.88 | 0.79 |
| 9 | 0.19 | 0.20 | 0.95 | 0.85 |
| 10 | 0.12 | 0.10 | 1.64 | 1.59 |
| 11 | 0.13 | 0.13 | 1.69 | 1.65 |
| 12 | 0.21 | 0.21 | 1.84 | 1.75 |
| 13 | 0.18 | 0.17 | 1.67 | 1.73 |
| 14 | 0.14 | 0.14 | 1.78 | 1.72 |
| 15 | 0.15 | 0.11 | 1.85 | 1.67 |
| 16 | 0.11 | 0.10 | 1.16 | 1.13 |
| 17 | 0.07 | 0.09 | 1.06 | 0.93 |
| 18 | 0.08 | 0.10 | 0.98 | 0.97 |
| 19 | 0.15 | 0.16 | 1.30 | 1.35 |
| Mean | 0.19 | 0.18 | 1.49 | 1.44 |
| SD | 0.08 | 0.07 | 0.37 | 0.36 |

The difference between values of P and K determined by the two digestion methods was not significant ($P = 0.05$). SD is standard deviation of mean.

Results obtained with the proposed digestion method were compared to those obtained with Kjeldahl (for N) and triacid digestion methods (for P, K, Ca, and Mg). Regression analysis was used to determine simple correlations between the values of various elements determined by the proposed and standard methods. Range, mean and standard deviation (SD) of the elemental concentrations were worked out to compare the results. Differences in values of an element determined by the proposed and standard methods were subjected to test of significance.

## RESULTS AND DISCUSSION

### Analysis of Pigeonpea Plant Samples

Results on the analysis of pigeonpea plant samples for N, P, K, Ca, and Mg using the proposed sulfuric acid–Se digestion procedure and routine digestion procedures showed that there was an excellent agreement in the values of various elements by the proposed method and the routinely used digestion methods (Tables 1–3).

***Table 3.*** Comparison of Values of Ca and Mg Determined by the Sulfuric–Se Digestion and Triacid Digestion Procedures in Pigeonpea Plant Samples

| | Ca Concentration ($g\,100\,g^{-1}$) | | Mg Concentration ($g\,100\,g^{-1}$) | |
|---|---|---|---|---|
| Sample No. | Sulfuric Acid–Se | Triacid | Sulfuric Acid–Se | Triacid |
| 1 | — | — | 0.30 | 0.31 |
| 2 | 1.58 | 1.66 | 0.33 | 0.33 |
| 3 | 1.85 | 1.91 | 0.37 | 0.37 |
| 4 | 1.44 | 1.65 | 0.28 | 0.28 |
| 5 | 1.80 | 1.67 | 0.30 | 0.32 |
| 6 | 1.55 | 1.50 | 0.32 | 0.31 |
| 7 | 1.29 | 1.19 | 0.21 | 0.20 |
| 8 | 1.30 | 1.23 | 0.25 | 0.25 |
| 9 | 1.47 | 1.51 | 0.24 | 0.24 |
| 10 | 0.93 | 0.90 | 0.13 | 0.13 |
| 11 | 0.96 | 0.92 | 0.13 | 0.13 |
| 12 | 1.32 | 1.16 | 0.21 | 0.21 |
| 13 | 0.98 | 0.94 | 0.15 | 0.16 |
| 14 | — | — | 0.13 | 0.13 |
| 15 | 0.95 | 0.93 | 0.13 | 0.13 |
| 16 | 0.72 | 0.62 | 0.11 | 0.10 |
| 17 | 0.87 | 0.86 | 0.09 | 0.10 |
| 18 | 0.69 | 0.67 | 0.08 | 0.09 |
| 19 | 0.89 | 0.84 | 0.12 | 0.13 |
| Mean | 1.21 | 1.19 | 0.20 | 0.21 |
| SD | 0.37 | 0.40 | 0.09 | 0.09 |

The difference between the values of Ca and Mg determined by the two digestion methods was not significant ($P = 0.05$). SD is standard deviation of mean.

***Table 4.*** Relationships Between the Values of N, P, K, Ca, and Mg ($g\,100\,g^{-1}$) in Pigeonpea and Rice Plant Samples Determined by the Sulfuric Acid–Se Digestion and Routine Digestion Methods

| Element | Regression Equation | Correlation Coefficient (r) |
|---|---|---|
| Pigeonpea plant samples | | |
| N | Sulfuric acid–Se–N = 1.017 Kjeldahl-N + 0.005 | 0.998 (n = 19) |
| P | Sulfuric acid–Se–P = 1.05 Triacid-P − 0.05 | 0.977 (n = 19) |
| K | Sulfuric acid–Se–K = 1.00 Triacid-K + 0.06 | 0.984 (n = 19) |
| Ca | Sulfuric acid–Se–Ca = 0.91 Triacid-Ca + 0.14 | 0.976 (n = 19) |
| Mg | Sulfuric acid–Se–Mg = 1.00 Triacid-Mg − 0.002 | 0.996 (n = 19) |
| Rice plant samples | | |
| N | Sulfuric acid–Se–N = 1.03 Kjeldahl-N − 0.03 | 0.998 (n = 19) |
| P | Sulfuric acid–Se–P = 1.08 Triacid-P − 0.01 | 0.945 (n = 19) |
| K | Sulfuric acid–Se–K = 1.00 Triacid-K − 0.02 | 0.995 (n = 19) |
| Ca | Sulfuric acid–Se–Ca = 0.74 Triacid-Ca + 0.06 | 0.901 (n = 19) |
| Mg | Sulfuric acid–Se–Mg = 0.99 Triacid-Mg − 0.004 | 0.969 (n = 19) |

The correlation coefficients (r) obtained between the values of various elements determined by the sulfuric acid–Se digestion procedure and the regular digestion methods were high. The slope of the regression equations showing relationships between the values of various elements by the proposed method and routine procedures was close to one, further indicating high precision in determining N, P, K, Ca, and Mg in pigeonpea and rice plant samples (Table 4).

Comparison of the sulfuric acid–Se digestion procedure with routine methods showed that the proposed digestion method had similar standard deviation (SD) for the determination of various elements (Tables 1–3).

## Analysis of Rice Plant Samples

Results with rice plant samples also showed that the values of N, P, K, Ca, and Mg determined by the proposed procedure were similar and highly correlated to those obtained using the routine digestion methods (Table 4).

The range, mean and SD (standard deviation) for determination of various elements by the sulfuric acid–Se digestion technique were comparable to those obtained with the routine digestion methods, indicating that the proposed digestion method is equally precise in determination of N, P, K, Ca, and Mg (Table 5).

***Table 5.*** Comparison of the Sulfuric Acid–Se Digestion and Routine Digestion Methods for Determination of N, P, K, Ca, and Mg in Rice Plant Samples (Elemental Concentration Is Expressed as g $100\,g^{-1}$ Dry Wt)

| | | Sulfuric Acid–Se Digestion | | | Kjeldahl/Triacid Digestion | | |
|---|---|---|---|---|---|---|---|
| Element | No. of Samples | Range | Mean | SD | Range | Mean | SD |
| N | 19 | 0.48–3.10 | 1.19 | 0.68 | 0.51–2.99 | 1.19 | 0.66 |
| P | 19 | 0.08–0.26 | 0.13 | 0.05 | 0.07–0.23 | 0.13 | 0.04 |
| K | 19 | 1.40–3.16 | 2.15 | 0.51 | 1.44–3.25 | 2.15 | 0.50 |
| Ca | 19 | 0.15–0.30 | 0.22 | 0.05 | 0.14–0.34 | 0.22 | 0.06 |
| Mg | 19 | 0.18–0.37 | 0.25 | 0.06 | 0.17–0.37 | 0.26 | 0.06 |

The results presented on the analysis of pigeonpea and rice plant samples clearly indicate that the plant digests prepared using the sulfuric acid–Se mixture can be used for determination of N, P, K, Ca, and Mg in plant materials in a single digest. Another advantage of the digestion procedure lies in that it can be used for determination of N and P in plant samples using a Technicon Autoanalyser II.[5] The proposed method is simple, relatively safe and requires fewer chemicals.

## REFERENCES

1. Gorsuch, T.T. Destruction of Organic Matter. In *International Series of Monographs in Analytical Chemistry*; Pergamon Press: New York, 1970; Vol. 39.
2. Wolf, B.A. Comprehensive System of Leaf Analysis and Its Use for Diagnosing Crop Nutrient Status. Commun. Soil Sci. Plant Anal. **1982**, *13*, 1035–1059.
3. Hue, N.V.; Evans, C.E. *Procedures Used for Soil and Plant Analysis by the Auburn University Soil Testing Laboratory*; Agronomy and Soils Department Series 106; Alabama Agricultural Experiment Station: Auburn, AL, 1986.
4. Morais, J.F.V.; Rabelo, N.A. *A Simple Plant Tissue Digestion Method*; Document No. 12; EMBRAPA-CNPAF: Goiania, Brazil, 1986.
5. Technicon Industrial Systems, *Technicon Autoanalyser II Manual*; Industrial Method No. 218-72A; Technicon Industrial Systems: Tarrytown, NY, 1972.

6. Jones, J.B., Jr.; Wolf, B.; Mills, H.A. *Plant Analysis Handbook: A Practical Sampling, Preparation, and Interpretation Guide*; Micro-Macro Publishing, Inc.: Athens, GA, 1991.
7. Sahrawat, K.L.; Ravi Kumar, G.; Rao, J.K. Evaluation of Triacid and Dry Ashing Procedures for Determining Potassium, Calcium, Magnesium, Iron, Zinc, Manganese and Copper in Plant Materials. Commun. Soil Sci. Plant Anal. **2002**, *33* (1&2), 95–102.