Effect of Wet Decomposition Methods on the Determination of Zinc and Selenium in Rice Leaves and Grains Using Electrophoresis

Jenq-Yann Yang*, Mo-Hsiung Yang* and Te-Hsien Lin**

*Institute of Nuclear Science, National Tsing Hua University, Hsinchu, Taiwan, Republic of China
**School of Technology for Medical Sciences, Kaohsiung Medical College, Kaohsiung, Taiwan, Republic of China

A method is described which combines radiotracer techniques with paper electrophoresis to investigate the optimal decomposition conditions for zinc and selenium in rice leaf and grain samples. After administration of the respective nuclides of $^{65}$Zn and $^{75}$Se solutions to the rice, samples of the tested rice are harvested and decomposed with a nitric and sulfuric acid mixture. The completeness of decomposition is investigated by measuring the respective radionuclide-containing species in the decomposed samples by electrophoretic analysis. The results indicate that the Se-containing rice samples are more easily decomposed than those containing Zn under our conditions. Different decomposition effects are observed between a nitric and sulfuric acid mixture and one which includes hydrogen peroxide. Decomposition may be connected with the analytical error.

Keywords Wet digestion, zinc, selenium, rice leaf, rice grain, electrophoresis

Zinc deficiency is common to rice. For instance, Shaira disease in India, Akagare Type II in Japan, Taya-Taya and Apulapaya in the Philippines and Hadda in Pakistan are all different names of this deficiency in rice. Zinc toxicity may occur due to acidification or zinc contamination of some soils. Plant Zn concentrations are a reflection of the available Zn levels in soils. They also indicate the influence of soil environment. Thus, plant tissue analysis can be helpful in diagnosing or confirming Zn deficiency or toxicity.

Selenium has also been shown to be an essential trace element in various animal species. The role of selenium in the prevention of human nutritional deficiencies is complicated. Keshan disease is a cardiomyopathy with a high case mortality that usually affects Chinese children and women of child-bearing age who ingest food locally grown in areas with Se-deficient soil. Since rice is one of the important sources of selenium for man, the analysis of selenium in rice leaves and grains is of fundamental importance.

The analytical process most commonly used for the determination of trace elements in biological samples is by digestion of the sample matrix, followed by direct instrumental determination of the trace elements in the decomposed sample, or, alternatively, by separation and/or concentration of the trace elements from the decomposed sample and finally by instrumental determination of the isolated elements. The sources of error most probably encountered in this analytical procedure, aside from systematic errors inherent in the trace analysis, may be due to the matrix effect by the undecomposed organic substance in the instrumental determination step or to the incomplete recovery of trace elements in the preconcentration step from the decomposed sample solution. The decomposition process undoubtedly constitutes an important step which vitally influences the analytical accuracy of trace elements in the biological samples. Decomposition of the sample is in fact one of the most important steps in trace analytical techniques in which voltammetry and flame and hydride generation atomic absorption spectrometry are employed as the measurement methods. However, to what extent the matrix is destroyed by a specific decomposition method has seldom been quantitatively evaluated. Conventionally, when clear and colorless solutions are obtained, or when total recovery of some elements is obtained, it has been tacitly assumed that oxidation of the organic matter has been completed for practical purposes. However, such assumptions are not necessarily reliable in all cases.

In the previous study, we developed a method combining radiotracer techniques with paper electrophoresis to investigate the effectiveness of the decomposition process of $^{65}$Zn-labeled liver samples. The results revealed that, aside from the $^{65}$Zn, there also appears on the electrophoretic histogram one $^{65}$Zn-containing...
organic species under certain specific decomposition conditions. On the basis of this study, it was assumed that the appearance of only one peak corresponding to the $^{65}$Zn$^{II}$ ion in the electrophoretic histogram means total destruction of the sample matrix in the decomposition process, while the additional appearance of $^{65}$Zn-containing species other than $^{65}$Zn$^{II}$ ion indicates incomplete destruction of the sample. This assumption is, however, not conclusively proven, because some other species in the decomposed sample might also migrate equally as Zn$^{II}$ in paper electrophoresis.

In this study, the work was extended to investigate the behavior of zinc and selenium in rice leaf and grain by a Sjostrand reflux wet digestion process with two acid mixtures (HNO$_3$-H$_2$SO$_4$, HNO$_3$-H$_2$SO$_4$-H$_2$O$_2$). The effectiveness of decomposition under these specific conditions is revealed from the electrophoretic histograms. It can be expected that complete decomposition is achieved if there appears on the histogram only one peak corresponding to the specific ions of the elements $^{65}$Zn$^{II}$ and $^{75}$Se$^{VI}$. The appearance of additional peaks in the histogram may thus indicate the presence of undecomposed rice species. The existence of any organically bonded species other than the specific ions in the decomposed sample solutions might decrease the recovery of that specific element in the separation step and thus result in analytical error.

### Experimental

#### Labeling with radioactive isotopes

The radioisotopes of $^{65}$Zn (half-life 245 d) and $^{75}$Se (half-life 120.4 d) were obtained by irradiating 100 mg spectroscopically pure metals or compounds in the reactor of National Tsing Hua University (THOR) at a flux of $1.6 \times 10^{13}$ n cm$^{-2}$ s$^{-1}$ for 60 h. The irradiated metals or compounds were dissolved in concentrated HNO$_3$, and the mixture was evaporated to dryness. Water was added and the resultant solution finally adjusted with NaOH to pH 2 - 3.

The young rice plants (30 d) were moved outdoors and shielded by lead bricks. The solution of $^{65}$Zn and $^{75}$Se was sprayed on the soil. The radioactive solution was sprayed two more times while the rice plants were growing for 3 months. The rice was harvested after a total period of 120 d, and the leaves and grains were prepared for the acid digestion.

#### Decomposition procedures

The radioisotopically labeled rice samples (each about 1.0 g) were digested with various wet oxidation methods with a mixture of concentrated HNO$_3$ and H$_2$SO$_4$ solution. A Sjostrand type of wet oxidation reflux apparatus was used, and the digestion performed followed basically the procedure established in our previous work.$^{6,7}$ The sample was put into the digestion flask (150 ml), and a mixture of 6 ml HNO$_3$ and 2 ml of H$_2$SO$_4$ with or/without 2 ml H$_2$O$_2$ was added to start the digestion. In the initial stage, reflux was continued with the condensate returning to the flask for about 15 min. Then the tap on the condensate reservoir was closed, and water and HNO$_3$ were collected in the condensate reservoir. When a slight darkening of the digest occurred, small amounts of condensate were allowed to drain into the flask to continue the oxidation and remove the darkening. The operation was continued until darkening no longer occurred and the white fumes of sulfur trioxide were observed. The whole process described above constitutes one-cycle digestion. Repeating the process two or three times constitutes the so-called two-cycle and three-cycle digesting processes.

After decomposition, the sample solution was adjusted with NaOH solution to about pH 6.5 for electrophoretic analysis.

#### Electrophoretic analytical procedure

Paper electrophoresis was conducted with a Camag high-voltage electrophoretic apparatus, as in our previous report.$^{6}$ About 50 µl aliquots of sample solutions were spotted on 2 x 48 cm strips of Whatman 3-mm filter paper that had been previously wetted in acetate buffer (pH 6.5, 0.25 M NaOAc-0.25 M HOAc). A potential of 1000 V was applied for 30 min. The distributions of radioisotopes in the paper strips cut into numbered 1-cm pieces were then measured with a well-type NaI(Tl) scintillation counter.

#### Solvent extraction procedure

Parts of decomposed sample solutions were subjected to solvent extraction for separating Zn$^{II}$ ion using 2% ammonium pyrrolidinedithiocarbamate/chloroform (APDC/CHCl$_3$) as extractant with a pH between 2 and 3. For separation of free selenium which is supposed to be in the Se$^{VI}$ form from the decomposed sample matrix by solvent extraction, prior reduction of Se$^{VI}$ to Se$^{IV}$ is necessary. The production was made by adding 25 ml of 6 M HCl to the digested sample solution and heating to 95°C for 1 h.$^{6}$ After reduction, the solution was diluted to 50 ml with water and finally extracted with APDC/methyl isopropyl ketone (APDC/MIBK) (50:50) at pH 1.5 - 2.5 to separate Se$^{IV}$.$^{9}$

### Results and Discussion

Wet digestion of biological materials with various methods and various acid mixtures has been extensively discussed in the literature. In our previous study, the Sjostrand reflux apparatus, pressure bomb digestion and low-temperature oxygen plasma asher were used.$^{6,7}$ Our results showed that the recovery yields of the one-cycle reflux digestion process with HNO$_3$-H$_2$SO$_4$ acid mixture for cobalt, copper, selenium and zinc in liver samples were 99.5, 93.4, 98.9 and 83.7% respectively, indicating Co and Se-containing liver samples are more easily decomposed than those containing Cu and Zn under our conditions. Among the liver samples tested,
the samples containing zinc are the most difficult to digest by wet oxidation; basically, three-cycle digestion is needed to ensure complete release of zinc from the liver matrix. In this study, we also pre-tested the liver sample and subjected it to one-cycle reflux digestion with HNO₃-H₂SO₄-H₂O₂ mixture, we found that the recovery yield of Zn can be increased to 92.8%. The effectiveness of the decomposition for different matrices may be quite different. In the present study, we therefore extend our work and digest some botanical samples—rice leaves and rice grains with these two acid mixtures.

To begin this study, the paper electrophoretic behavior of the respective ions of Zn²⁺ and Se⁴⁻, the probable forms expected to be present in the digested sample solution, are first investigated by the procedures described in our previous work.⁶ ⁷ The electrophoretic histograms of the respective elements indicate that the peaks corresponding to Zn²⁺ in the acetate buffer of pH 6.5 appeared on the negative side about 12 - 14 cm from the zero point, and that of Se⁴⁻, which is known to be an oxyanion, appeared on the positive side about 13.5 cm from the zero point under this specific analytical condition. For a rice sample, if it is not completely digested, there should also be present other species containing the element of interest aside from the ionic forms described above. The separation of the totally decomposed species, typically as free ionic forms in the digested sample, can be basically achieved by the proper choice of electrophoretic conditions. However, the question to be clarified is whether the species identified as an undecomposed fraction appearing on the electrophoretic histogram is really an incompletely decomposed species or a species resulting from recombination of once-free ions with the organic residues in the subsequent treatment of decomposed sample solution. To verify this, an experimental design was made by mixing the respective ions of ⁶⁵Zn²⁺ and ⁷⁵Se⁴⁻ with the decomposed rice leaf and grain sample solution containing no radioactive tracer and then subjecting the mixture to electrophoretic analysis. The results so obtained indicated that, aside from the peaks corresponding to the respective ions of ⁶⁵Zn²⁺ and ⁷⁵Se⁴⁻, there appear no other perceivable peaks in the histograms. This excludes the possibility of formation of radioactively labeled species due to isotope-exchange reaction and consequently suggests that any radioactively labeled species other than the ionic form appearing on the histogram result directly from the undecomposed rice material.

On the basis of the preliminary results obtained, the use of electrophoretic analysis to study the optimal decomposition conditions of ⁶⁵Zn and ⁷⁵Se-labeled rice samples was considered feasible. In the following sections, the results for the respective elements are described.

**Zinc**

Zinc deficiency symptoms common to rice are: Lower leaves have chlorotic midribs particularly towards the base. The leaves develop brown rusty spots which coalesce and form continuous areas. In the case of acute deficiency, the whole leaf becomes brown and dry and plants may succumb. A zinc deficient rice field may give a brown rusty appearance and the stand of rice is uneven. Diagnosis of Zn deficiency from symptoms appears easy, but in practice it is not. Zinc deficiency may depress rice yields by as much as 50% without producing any symptoms.¹ The diagnosis based upon symptoms becomes uncertain if multi-nutrient deficiencies occur simultaneously. Thus, suspected deficiencies or toxicities should always be confirmed by chemical analysis.

Figure 1 shows the electrophoretic histograms of the rice leaf sample treated with ⁶⁵Zn when the sample subjected to varying extents of wet-oxidation digestion with HNO₃-H₂SO₄ mixture. As seen from the figure, the peak appearing on the negative side about 13 cm from the zero point corresponds to the ⁶⁵Zn²⁺ species, while the peak appearing at about 1 cm from the zero point on the same side as Zn²⁺, corresponding to a slightly positively charged species, can be assumed to be an incompletely dissociated Zn-containing species. It is seen that the percentage yields of ⁶⁵Zn-containing organic species decrease with increasing the digestion cycle; they are 11, 3.5 and 0.3%, corresponding respectively to one-cycle, two-cycle, and three cycle digestion processes. It is interesting to explore more deeply the fate of the undissociated species in the course of separation by the commonly employed solvent extraction method. In this study APDC/MIBK is used as extractant, to separate Zn²⁺ ion from the digested sample solution. Interestingly, the percentage of ⁶⁵Zn extracted into the organic phase very closely agreed with the fraction of ⁶⁵Zn²⁺ shown in
the histogram of Fig. 1, while that remaining in the aqueous phase is found equivalent to the fraction of $^{65}$Zn-organic species. The percentages of extraction for those three samples with different extents of decomposition are also shown in the same figure for ease of comparison. The experimental facts show that one of the sources of analytical error is the incomplete dissociation of sample matrix, as is obvious from the example shown in Fig. 1: about 11% of error might be expected for the one-cycle digested sample, due to the apparently low recovery of Zn by the solvent extraction process.

Figure 2 shows the results of the rice leaf sample subjected to varying extents of wet-oxidation digestion with HNO$_3$-H$_2$SO$_4$-H$_2$O$_2$ mixture. The results are very similar to those shown in Fig. 1, except that the digestion effectiveness is much more complete. For example, the percentage yield of $^{65}$Zn-containing organic species is 4.4% versus 11% in Fig. 1 for one-cycle digestion.

Figure 3 shows the results of the rice grain sample subjected to varying extents of wet-oxidation digestion with HNO$_3$-H$_2$SO$_4$-H$_2$O$_2$ mixture. The results are also similar to those shown in Fig. 1. The rice grain sample is more easily decomposed than the rice leaves. Only 2.7% of $^{65}$Zn-containing organic species was found after one-cycle digestion and about 1% was found after two-cycle digestion.

**Selenium**

In New Zealand, where the soil Se content is extremely low, the Se content of herbage is also low, and deficiency diseases in sheep and cattle, once a serious problem, are prevented by frequent Se drenching.$^{10}$ In mainland China, the Se content in food grain is closely related to the Keshan disease, namely, the difference of the Se content in food grain between the disease belt and the disease-free belt is very significant.$^{11}$ The determination of Se content in plants and one of the steps—digestion are therefore of importance.

In the present study we used an HNO$_3$, H$_2$SO$_4$ and H$_2$O$_2$ mixture as the digestion solution to investigate the behavior of selenium in rice samples as regards the digestion process. Figures 4 and 5 show the histograms of the samples subjected to reflux digestion. It is seen that in both figures there appears a predominant peak on the positive side about 13.5 cm from the zero point and a small peak standing near the zero point. The negatively charged species is presumably a selenate ion resulting from the decomposition of the rice sample in the highly oxidative digestion solutions, while the neutral species standing on the zero point is the undecomposed matrix species. To prove that the fraction contains Se$^{IV}$, the digested sample solution was subsequently heated in 6 M HCl. After reduction, the Se$^{IV}$ extractable by APDC/MIBK was found to be very closely equivalent to the $^{75}$Se fraction that appeared on the positive side of the histograms.

The controversy about the inconsistent results for selenium determination in various rice samples can be basically attributed to incomplete conversion of native forms of selenium such as selenomethionine to selenite-selenate, loss of selenium during the digestion process, and the state or form of selenium that is not detectable by the analytical technique employed.$^{7}$ From the results it is clear that selenium can be almost completely released from the rice matrix into ionic form as selenate after one-cycle digestion process. It seems that selenium in rice
matrices is not so difficult to digest as previously regarded. The reported inconsistency for Se determination most likely is due not to decomposition efficiency but to the inadequacy of the analytical techniques employed for the determination of selenium. Even if the trace elements have been completely released from the rice matrix, there may still remain various types and amounts of organic residues in the digested sample solution. Unlike techniques such as atomic absorption spectrometry (AAS) and inductively coupled plasmaatomic emission spectrometry (ICP-AES), which rely on atomization of the element from solution and are not so prone to organic interferences, stripping voltammetry is more susceptible to the interferences. The organic residue can foul up the electrode by blockage or interference mechanisms of the adsorption type, and analytical errors may thus result from this electroanalytical technique.

In conclusion, the method which combines radiotracer techniques with electrophoresis provides a unique way to study the relative effectiveness of the decomposition process of biological samples. In our laboratory, the radioactive-labeled liver, rice leaf and grain samples of respective nuclides of $^{65}$Zn and $^{75}$Se have so far been prepared and subjected to wet-oxidation digestion with the acid mixture of HNO$_3$–H$_2$SO$_4$ and HNO$_3$–H$_2$SO$_4$–H$_2$O$_2$. The results show that the rice leaf and grain samples can be more easily decomposed than liver sample under the same decomposition condition, and that addition of H$_2$O$_2$ as an oxidant to the digestion solution of HNO$_3$–H$_2$SO$_4$ can effectively facilitate the decomposition of sample matrices.

References


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