

with the larger losses encountered under field conditions through leaching and the stress of higher yields. To adjust the particular glass for single-season use, reactivity would need to be increased by grinding to a higher degree of fineness.

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## PLANT ANALYSES

# Analysis of Dried Plant Material by X-Ray Emission Spectrograph

A method is described for determining the concentration of a number of elements in dried, ground plant material using an x-ray emission spectrograph. The concentration of a given element is determined by using the ratio of radiation intensity of that element to the radiation intensity of scatter. Working curves are developed using plant material which has been analyzed chemically. The technique seems to correct for most of the day-to-day variations to which such an instrument is subject. However, a consistent program of daily reference standards is necessary for the satisfactory, continuous operation of the instrument.

RECENT REVIEWS have pointed to the increasing use of the x-ray emission spectrograph as an analytical tool in the fields of metallurgy, ore and mineral analysis, petroleum technology, quality control, and, to a limited extent, in biological assays (2, 9, 10). The simplicity of the spectra and the relative ease with which determinations of certain elements can be made suggest its application to the analysis of plant material.

This paper describes a technique which has proved useful for the quantitative and semiquantitative analysis of dried, ground plant material without ashing or concentrating. One of the chief problems in applying the x-ray emission technique is the matter of matrix composition and its effect on the line intensity. The successful use of an internal standard (3, 8, 11) and of dilution (3, 6) to overcome these effects has been reported. The method reported here uses, in effect, the scatter from the sample as an internal standard. While the basis on which the method developed was different, it is similar to the suggestion of Kemp and Andermann (7).

#### Equipment

A Norelco x-ray spectrograph with an FA-60 x-ray tube is used. The exit port of the sample irradiation chamber is replaced with a  $1/8 \times 4$  inch Soller slit assembly. For the lower atomic number elements, a helium path, sodium chloride crystal,  $0.020 \times 4$  inch Soller slit assembly, and No. 62030 GM tube—to detect

soft radiation—are used. For the elements of atomic number greater than 25, a lithium fluoride crystal,  $0.005 \times 4$  inch Soller slit assembly, scintillation counter, and scintillation-proportional unit (a linear amplifier and fast scaler) are used.

The sample holder is modified so that an aluminum alloy frame, 0.05 inch thick, can be slid into the holder and supported along the edges only. The center of the frame is cut out to match the opening in the holder. One side of the frame is covered with 0.00025-inch Mylar film stretched tight and cemented to the frame with rubber cement. The irradiation beam is carefully centered so that no part of the holder or frame is irradiated.

#### Method

Samples of plant material are dried at 70° C. in a forced hot-air oven for 48 hours and ground in a Wiley mill to pass a 20-mesh stainless steel sieve as previously reported (5).

Subsamples are placed in the frame and leveled with the top. Care is taken to distribute the sample as uniformly as possible with a minimum of segregation and packing of particles. It is not necessary to weigh definite amounts of the sample, nor is it desirable to pack the sample into the frame.

For quantitative analysis, the line-to-scatter ratio for a given element—i.e., the angle at which the goniometer is set to receive the radiation from the element being measured—is used as a measure of its concentration. The scatter refers

to the radiation intensity in a region free of lines. An arbitrary angle is selected such that the scatter intensity as measured will be as nearly as possible representative of that at the line angle if there were none of the element in the sample. The scatter measures all the factors of the sample other than concentration of the element being measured.

The goniometer is set at the desired angles and the time for a preset number of counts is recorded. These data are converted to counts per second, the line-to-scatter ratio calculated, and the concentration read from the working curve for that element. The working curve is prepared by obtaining similar data on samples of dried, ground plant material which have been analyzed chemically (5, 12), plotting the line-to-scatter ratio as a function of the concentration of the element in the plant material and fitting a straight line to the data by the method of least squares.

For semiquantitative analysis, the line-to-scatter ratios are calculated from the intensities measured on the scan and the concentrations read from the working curve used for quantitative analysis. The scans are obtained by setting the goniometer to run automatically over the desired range at 1° per minute and the chart recorder to run at 0.5 inch per minute.

#### Results

Following the above procedure working curves for four elements were established (Table I). The constants *a* and *b*

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**Table I. Wave Lengths and Working Curve<sup>a</sup> Constants for Individual Elements**

Element and Line	Wave Length, A.		Working Curve Constants <sup>b</sup>			Correlation with Chemical Determinations		Mean Deviation from Working Curve <sup>c</sup>		Working Sensitivity, P.P.M.
	Fluorescent line, F	Scatter radiation, S	a	b	b'	F/S	F - S	F/S	F - S	
Mn K $\alpha$	2.103	2.044	0.982	0.0064	156	0.998	0.963	19.7 $\pm$ 20.5	28.9 $\pm$ 38.0	10
Co K $\alpha$	1.791	1.860	1.128	0.0201	49.8	0.999	0.943	2.76 $\pm$ 2.44	9.28 $\pm$ 11.87	3
Zn K $\alpha$	1.437	1.408	1.013	0.0169	59.2	0.997	0.984	1.43 $\pm$ 1.09	3.71 $\pm$ 2.70	3
Mo K $\alpha$	0.710	0.727	1.030	0.0027	370	0.990	0.978	1.61 $\pm$ 1.26	2.49 $\pm$ 1.85	10

<sup>a</sup> Based on 15 to 20 samples.

<sup>b</sup>  $F/S = a + bC$ .  $b' = 1/b$ . These values are peculiar to the particular instrument used to obtain them.

<sup>c</sup> Mean with standard deviation of the absolute differences between the concentrations determined chemically and by the fitted curve from the x-ray spectrographic data.

are the intercept and slope of the respective curves for this specific arrangement. They will not necessarily apply to any other instrument. The minimum concentration of each element, which experience showed can be determined with confidence, is shown as "working sensitivity" in the last column of Table I. Except for cobalt, the sensitivity is adequate for all practical purposes in nutrition research.

The reproducibility of the method is shown in Table II. Two samples of alfalfa were selected for the test. Subsamples from these were analyzed chemically for zinc (72). Other subsamples were taken and presented to the x-ray spectrograph on several different days. Zinc concentrations read from the working curve are reported, together with the chemical data, in Table II. The deviation in both methods is of the same order.

**Discussion**

The radiation observed at any given goniometer setting consists of three components: a background, including stray radiation from the x-ray tube and sample, which does not satisfy the Bragg relation; a scatter radiation, from the primary beam and its interaction on the sample, which satisfies the Bragg relation; and a fluorescent (characteristic) radiation, from the components of the sample, which satisfies the Bragg relation.

The level of background is mainly an instrument design factor. With the equipment used, this component is sufficiently consistent and low to be ignored in the following considerations. The intensity of the scatter radiation is depend-

ent upon the operating conditions of the x-ray tube as well as the nature, composition, and thickness of the sample; it will vary with goniometer setting. The intensity of the fluorescent radiation of a line will depend upon the concentration of the element in the sample as well as the operating conditions of the x-ray tube and nature, composition, and thickness of sample.

Neglecting the background component, the observed intensity of a given fluorescent line,  $F$ , of an element should be related to its concentration,  $C$ , in the sample by

$$F = S_f + \phi_f C \quad (1)$$

where  $S_f$  is the intensity of radiation due to scatter at the goniometer setting for the fluorescent line, and  $\phi_f$  is a proportionality coefficient. If, for a given set of operating conditions,  $S_f$  and  $\phi_f$  do not vary from sample to sample, a plot of  $F$  vs.  $C$  should yield a straight line. If the change in  $C$  is sufficient to alter the absorptive nature of the sample, then  $\phi_f$  becomes a function of  $C$  but the plot, while no longer linear, is still usable as a working curve. In certain applications of the technique, these conditions apply (2, 4).

In the case of ground plant material, either as described or in pellets, no consistent relationship was obtained between the observed intensity,  $F$ , of a fluorescent line and the known concentration,  $C$ , of that element. The scatter radiation intensity,  $S_f$ , in the region of the line varied greatly from sample to sample. By using the scatter radiation intensity near the line as an estimate of  $S_f$  and plotting  $(F - S_f)$  vs.  $C$ , marked improvement was obtained as shown in Figure 1. The variation in the concentration of carbon, oxygen, potassium, and calcium in plant material will produce the only marked variation in the absorption coefficient of the sample for the molybdenum  $K\alpha$  line and this variation in the absorption coefficient cannot be the reason for the deviations still apparent in Figure 1.

The term  $\phi_f$  is a function of the operating conditions of the x-ray tube and the nature, composition, and thickness of the sample (7). The scatter radiation will also be a function of these variables, and perhaps, under certain conditions, of the same form. If this reasoning is correct, it may be possible to find a region free of fluorescent lines from the sample

**Table II. Comparison of Zinc Concentrations in Two Samples of Alfalfa as Determined by Chemical and X-Ray Emission Spectrographic Methods**

Sample	Chemical Method <sup>a</sup>	X-Ray Emission Spectrographic Method <sup>b</sup>
A	18.0 $\pm$ 0.25	18.3 $\pm$ 1.70
B	30.3 $\pm$ 0.87	28.7 $\pm$ 0.69

<sup>a</sup> Means and standard deviations of five subsamples and duplicate determinations.

<sup>b</sup> Means and standard deviations of five subsamples and duplicate determinations made on different days.

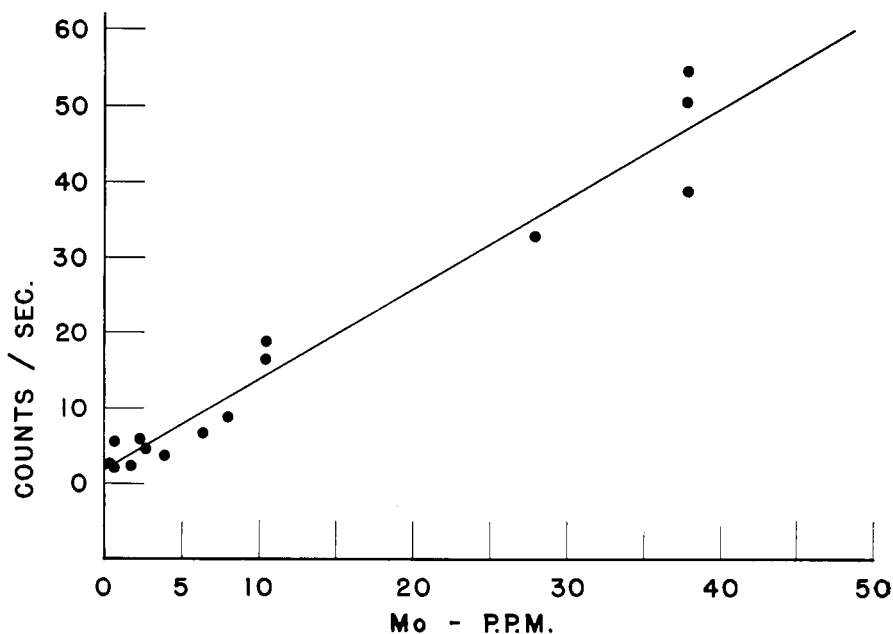


Figure 1. The intensity of the molybdenum  $K\alpha$  radiation minus the estimated intensity of the scatter radiation as a function of the molybdenum content of dried, ground plant material

and to measure a scatter radiation,  $S$ , differing from  $S_f$ , such that dividing Equation 1 by  $S$  yields

$$F/S = a + bC \quad (2)$$

where  $a$  and  $b$  are constants over the range of uncontrolled variables encountered in practice.

The nonrigorous, intuitive development of this linear form is intended to show only that, under certain conditions, a relation of this form may be approached. The practical result of this hypothesis is shown in Figure 2. Using the same materials as in Figure 1 and selecting an angle of  $20.80^\circ 2\theta$  (0.727 Å.) for the determination of  $S$ , the plot of  $F/S$  vs.  $C$  is shown to be linear. The data presented in Figure 2 represent 15 samples of plant material from a variety of species and locations.

To compare the working curves in Figures 1 and 2, correlation coefficients were determined for the relationships between  $F/S$  and molybdenum concentration as determined by chemical analysis and for that between  $F - S$  and the concentration of molybdenum. The results shown in Table I indicate either system is adequate but in considering all elements tested,  $F/S$  is preferable to  $F - S$ . To examine further  $F - S$  and  $F/S$ , the deviations from each curve were calculated (Table III). Either method of preparing a working curve will give satisfactory results. However, the standard deviation for the  $F/S$  differences is somewhat less than that for  $F - S$ . The mean and standard deviations for all four elements are shown in Table I and support the conclusion that the use of the  $F/S$  system may be advantageous.

As the working curves are straight lines, the equation for the line can be used to calculate  $C$ . Equation 2 is transposed, solving for the concentration permitting machine calculation of the concentration from the intensity measurements. The working curve constants so far developed for this system are given in Table I. The slope term  $b'$  (reciprocal of  $b$ ) is a measure of the sensitivity of the method—i.e., the concentration in parts per million equivalent to 1 unit of the ratio  $F/S$ . As a rule, two intensities in the ratio are estimated by fixed counts in excess of 25,000; thus the counting error in the ratio will be less than 1%. The ultimate sensitivity of the method, then, is 1 to 2% of  $b'$ . The measure of ultimate sensitivity is modified by a factor based on experience of sampling error and other sources of variation.

The measure of interest in this relation is the ratio of the fluorescent intensity to a scatter intensity. This suggests the scatter should arise only from the sample. To prevent scatter from other than the sample, the sample must be self-supporting or infinitely thick. As the development of Equation 2 suggests the

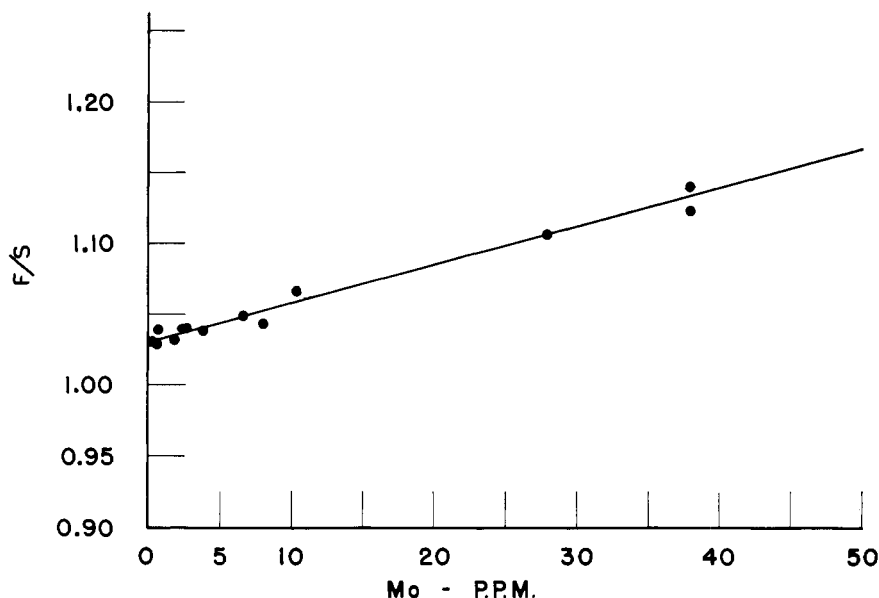


Figure 2. The ratio of the molybdenum  $K\alpha$  radiation intensity to the scatter radiation intensity as a function of the molybdenum content of dried, ground plant material

Table III. Comparison of  $F/S$  with  $F - S$  for Determining Molybdenum Concentration in Plant Tissue

Species and Location	Chemical Analysis $C_1$	Determined from $F/S$ Curve $C_2$	Difference	Determined from $F-S$ Curve $C_2$	Difference
Alfalfa stems, New York	0.3	0	0.3	0.8	0.5
Fern, Utah	0.6	-0.7	1.3	2.6	2.0
Alfalfa leaves, New York	0.6	2.6	2.0	3.1	2.5
Aster, Utah	1.7	0.4	1.3	0.3	1.4
Alfalfa stems, Nebraska	2.3	3.3	1.0	3.4	1.1
Lupine, Utah	2.7	2.6	0.1	2.3	0.4
Sedge and grass, Utah	3.8	2.6	1.2	1.7	2.1
Alfalfa stems, California	6.4	6.2	0.2	4.2	2.2
Astragalus, Utah	8.0	4.4	3.6	5.9	2.1
Alfalfa leaves, California	10.4	12.8	2.4	12.1	1.7
Alfalfa, Utah	10.4	12.8	2.4	14.1	3.7
White clover, Nevada	28.0	27.8	0.2	25.9	2.1
Sweet clover, Nevada	38.0	39.5	1.5	43.9	5.9
Strawberry clover, Nevada	38.0	33.6	4.4	30.8	7.2
Sweet clover, Nevada	38.0	40.2	2.2	40.4	2.4
Mean and standard deviation			$1.61 \pm 1.26$		$2.49 \pm 1.85$

maximum sensitivity is obtained with thin samples, a compromise for an essentially nonscattering backing was made. Mylar, a plastic film, is available in 0.00025-inch thickness, representing approximately 100  $\gamma$  per sq. cm., yet having considerable tensile strength. This material then serves as an essentially nonscattering backing for the sample.

As the scatter component includes higher orders of short wave length scatter from Equation 2, one would expect that maximum sensitivity for the long wave length lines would be with thin samples. To investigate the influence of sample thickness or weight, frames differing in thickness were prepared. The tared frames were filled with subsamples from a single reference sample, weighed, and the sample weight per unit area cal-

culated. Intensity measurements for manganese, zinc, and strontium were made and the ratios of  $F/S$  were calculated. The ratios were normalized to 1.00 at a sample weight equivalent to 34 mg. per sq. cm., which appeared to be the point of maximum sensitivity for manganese. These data are plotted as arbitrary units of  $F/S$  vs. sample thickness (Figure 3). The data up to approximately 35 mg. per sq. cm. agree with this theory.

The inflection point at this thickness is difficult to explain. The reasoning would anticipate such an inflection due to the sample mount. However, the mount in this case is hardly thick enough to produce the inflection at the observed thickness. There is also the possibility that in the scatter radiation there is a purely

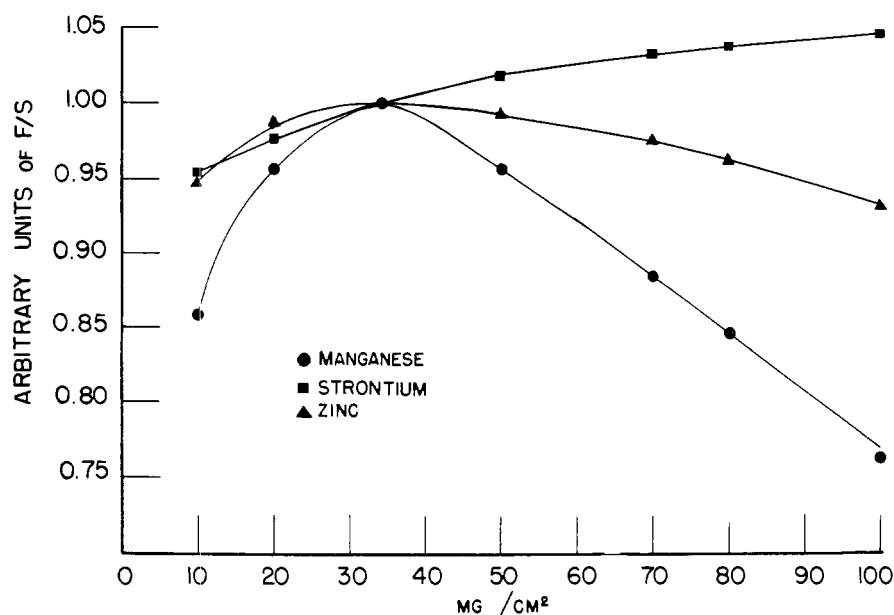


Figure 3. The ratios (in arbitrary units) of the  $K\alpha$  radiation intensities to the scatter radiation intensities as a function of the weight of the sample

surface reflection phenomenon not affected by thickness. This hypothesis would explain the decrease in sensitivity with samples thinner than 35 mg. per sq. cm. To explain such a consistent point of inflection, such a surface reflection phenomenon would have to be rather independent of the variation in the nature of the sample.

On the basis of these, a 0.05-inch thick frame was chosen, which for most materials yields samples equivalent to 30 to 40 mg. per sq. cm. Figure 3 also indicates the range of sample weights over which the method is essentially constant permitting the use of a fixed frame thickness without weighing the sample.

This method appears to correct for many of the day-to-day variations in the instrument as well as the sample variables. However, a consistent program of daily reference standards is necessary to keep check on these instrument variables. For this purpose, aluminum alloy samples that give lines over the range of interest are recommended. The daily position of these lines on the goniometer scale and their intensity under standard conditions are logged and serve as a check on instrument stability.

The degree of deviation from the standard values which can be tolerated depends upon the accuracy desired. Generally the goniometer is not realigned

until the peaks deviate about  $0.1^\circ$ . Correction factors are applied to the desired angles, however. Also realignment for intensity is not made unless deviations in excess of 5% of the standard rate are obtained. These same standards are used to realign to the theoretical angles and standard intensities. Any rugged standard material which will give lines in the area of interest that are measurable under the power settings required for the samples will suffice.

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## FROZEN FOODS STORAGE EFFECTS

### Effect of Storage Conditions on Nutrients in Frozen Green Beans, Peas, Orange Juice, and Strawberries

PROPER QUICK FREEZING of foods is effective in preserving their nutritional value. To provide basic data on nutrients in commercially available frozen fruits, juices, and vegetables, an extensive survey was carried out on samples taken from regular production lines (7). Much information is available on the effects of maltreatment, during processing of frozen foods, on certain nutrients—particularly ascorbic acid (6). Although direct observations were not made in this regard, the nutrition survey data indicated that, in general, maltreatments which can cause serious loss of nutrients in harvesting and processing are avoided.

Frozen foods are known to remain well

preserved at  $0^\circ$  F., but storage at higher temperatures can cause vitamin losses and marked effects on color and flavor, which results in lower quality at the retail level. Although surveys have suggested that less than 1% of frozen food products are subjected to serious temperature abuse during commercial handling (5), it is important to collect information on stability under various storage conditions. The Department of Agriculture is conducting a broad study on time-temperature effects (7). The nutrition survey samples referred to above were stored for an average of approximately 7 months at  $0^\circ$  F. before they were analyzed. The present report deals with nutritional values in frozen

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green sweet peas, French-style green beans, sliced strawberries, and orange juice concentrate after storage under less favorable conditions. Twenty-one nutrients were determined in samples stored for periods ranging up to 11 months at  $0^\circ$  F. with an additional 1-month exposure at  $20^\circ$  F. in each case.

#### Materials and Methods

Two cases of each product were obtained directly from packers. Each product sample was of uniform good quality and processed from the same lot of raw material. The strawberries, French-style green beans, and green sweet peas were packed during the 1954 season. Concentrated orange juice