Sample Preparation for Portable XRF Analysis of Plant Samples

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Introduction

Quantitative analysis of plants is a crucial aspect of crop management because it shows the concentration of nutrients that are present. This can indicate if there are any nutrient imbalances, toxic levels of nonessential elements, or any uptake deficiencies in the plant.

The nutrients themselves are in the form of pure elements, some of the most common ones being nitrogen (N), magnesium (Mg), phosphorus (P), sulfur (S), potassium (K), calcium (Ca), manganese (Mn), iron (Fe), copper (Cu), and zinc (Zn).

Plants tend to be heterogenous, thin (i.e. leaves), and contain substantial amounts of water, none of which are ideal for Handheld XRF (HH-XRF) analysis.

With the recent release of the Bruker Plants Calibration for portable X-ray fluorescence (pXRF), it is important to establish consistent preparation techniques for plant samples to attain consistent results.

Bruker's Plants calibration is optimized for dried, crushed, and packed plant samples. To account for any variation in matrix or density, the calibration uses Compton normalization, which normalizes the data to the Rh(K) tube scatter peak. See Table 1 for elements and their LODs.

Method

An individual leaf is too thin for the X-ray beam to efficiently fluoresce because most of the X-rays are penetrating through the leaf, resulting in a proportionally lower X-ray signal. Another issue is the fact that in the case of spinach, up to ninety-five percent of the overall mass can be from water. In XRF, the presence of water will act as an X-ray attenuator, which will lower the quantitative results.

Element	LOD	Element	LOD
Mg	993	Ni	2
Al	119	Cu	1
Si	59	Zn	1
P	15	As	2
Si	12	Se	2
Cl	24	Br	2
K	35	Rb	3
Ca	45	Sr	3
Ti	3	Mo	3
V	1	Cd	18
Cr	3	Ba	147
Mn	3	Hg	3
Fe	2	Pb	6

Table 1. LOD for elements in the plant calibration, not accounting for any elemental peak overlaps

This preparation requires about three to four grams of final dried plant material per sample. Since spinach is comprised of up to ninety-five percent water weight, approximately forty grams of fresh spinach leaves is required to start with. Air-drying is ideal, but can be time consuming. The best method proved to be placing the leaves on a heat-safe mat, and placing that on a large hot plate on low setting. Using this method, the leaves were fully dried overnight.

A food processor can break the dried plant material down into a coarse powder, and a mortar and pestle can be used to grind it down finer. A food strainer can be used to separate unbroken pieces. The goal is to get a relatively uniform consistency and homogenize the sample as in Fig 1a.

Using an open-ended 42mm XRF sample cup, cover one side with 3µm Prolene™ film, and clamp it with the O-ring. Fill the cup with three to four grams of the plant powder. Pack the powder down into the cup (careful not to puncture the Prolene™). The packed layer should be about fifteen to twenty millimeters thick to ensure that the sample is infinitely thick to the X-ray. Stuff with a cotton ball to keep it packed, and seal with the lid. The sample is now ready for analysis.

Figure 1a

Figure 1b

Figures 1a and 1b. Spinach leaves crushed and prepped for analysis; packed in cup

Results

To illustrate the impact that sample preparation has on the results, measurements were taken on five fresh spinach leaves before they were dried and prepared. Each fresh leaf was measured at five different locations to assess the "intra" leaf variation, with the leaf laid flat on the sample stage.

After these five randomly sampled leaves were measured, the spinach was prepared into the single XRF cup shown in Fig 1b. Five different points were then measured on the final prepared sample, with the instrument set up as shown in Fig 2. All measurements were taken using the Bruker Tracer 5i using the Plants calibration. This is a dual-phase calibration, measured for thirty seconds on each phase, for a total of sixty seconds.

Figure 2. TRACER 5i in desktop stand with sample under safety cover

Table 2 shows results for P, K, Ca, and Fe from five points on a single unprepared spinach leaf to show the variation within a single leaf.

Leaf 1	P	K	Ca	Fe
Pt1	0.020	0.756	0.098	0.004
Pt2	0.014	0.538	0.172	0.006
Pt3	0.022	0.679	0.146	0.005
Pt4	0.026	0.549	0.199	0.008
Pt5	0.025	0.717	0.183	0.005
Avg	0.021	0.648	0.160	0.006
StdDEv	0.005	0.099	0.039	0.001
RSD%	21.669	15.313	24.754	25.528

Table 2. Five points from a single fresh spinach leaf

Table 3 shows a five-point average from five different spinach leaves to show the variation between leaves. The results in Tables 2 and 3 show relatively low levels of each nutrient (all < 1%), which is expected in a fresh plant material. The relative standard deviation (RSD%) for the five points however, is quite poor for all elements. This suggests the sample is not very homogenous.

Averages	P	K	Ca	Fe
Leaf 1	0.017	0.785	0.100	0.004
Leaf 2	0.021	0.648	0.160	0.006
Leaf 3	0.021	0.667	0.244	0.007
Leaf 4	0.016	0.709	0.161	0.005
Leaf 5	0.014	0.758	0.122	0.004

Table 3. Averaged values from 5 fresh spinach leaves

Table 4 shows the five-point summary from the prepared sample. By comparison, the nutrient concentrations increased dramatically with the elimination of water, and the RSD% improved (nominally) by a factor of ten from the grinding and homogenizing.

Leaf 1	P	K	Ca	Fe
Pt1	0.477	7.922	0.880	0.017
Pt2	0.462	7.886	0.877	0.017
Pt3	0.483	7.998	0.877	0.016
Pt4	0.480	8.00	0.857	0.016
Pt5	0.463	7.836	0.872	0.017
Avg	0.473	7.928	0.872	0.017
StdDEv	0.010	0.071	0.009	0.000
RSD%	2.088	0.898	1.055	2.308

Table 4. Five points collected on the dried and prepared sample

Although the results increased after the sample was prepared, the relative change is not consistent. This is because the presence of water acts as an attenuator. As dictated by X-ray physics, "lighter" elements (lower atomic number) are more susceptible to attenuation. As the element becomes more energetic, the impact of the attenuator decreases. This is why phosphorus increase by 2106% relative, whereas iron only increases by 193% relative when comparing with their fresh results.

Figure 3 compares the average nutrient concentrations in the fresh leaves (blue) versus prepared sample (orange). The values highlighted in green shows the relative percent increase in the concentration for each nutrient after preparing the sample.

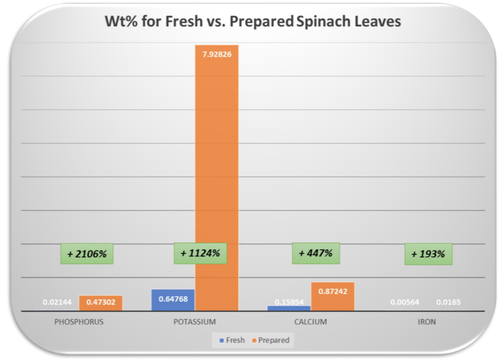

Figure 3. Fresh vs. dry nutrient concentrations and their relative percent changes

Conclusions

- Measuring fresh plant material is useful for qualitative analysis to determine if an element present or not
- Attaining quantitative information requires samples to be prepared, which is consistent with most operating procedures for plant analysis
- Bruker's Plants calibration provides a versatile calibration for a variety of plant materials to attain nutrient concentrations