Technical Note #2002

Sample Preparation of Plant Samples for Handheld XRF

Author: Andrew Lee, Application Specialist

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, magnesium, phosphorus, sulfur, potassium, calcium, manganese, iron, copper, and zinc.

PORTABLE XRF FOR PLANT SAMPLES

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. 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. This application note will explain preparation techniques, and compare the results from fresh unprepared spinach leaves with fully prepared dried leaves.

The 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. The following table shows the elements included in the Plants calibration, and their theoretical limits of detection (LOD).

Figure 1

Element	LOD	Element	LOD
Mg	993	Ni	2
Al	119	Cu	1
Si	59	Zn	1
P	15	As	2
S	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

LOD for elements in the Plant calibration, not accounting for any elemental peak overlaps.

SAMPLE PREPARATION METHODS

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.

1. Drying:
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.

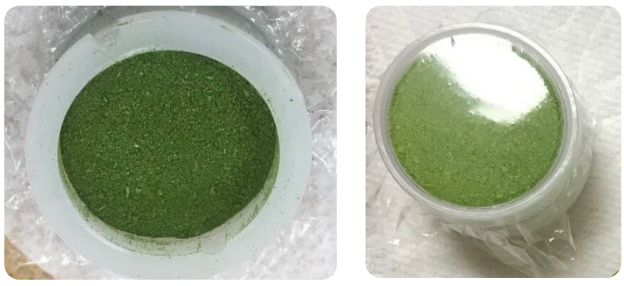
Figure 2

3.8 grams of dried spinach leaves, after losing about 95% of mass from water

2. Crushing & Homogenizing:
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 shown in Figure 3a.

Using an open-ended 42mm XRF sample cup, cover one side with 3um 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, as shown in Figure 3b.

Figures 3a and 3b



Spinach leaves crushed and prepped

MEASUREMENTS & ANALYSES

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 Figure 3b. Five different points were then measured on the final prepared sample, with the instrument set up as shown in Figure 4. 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 4

TRACER 5i with desktop stand measuring a prepared plant sample

IMPACT ON RESULTS – WET VS DRY

Figure 5 below shows results for Phosphorus, Potassium, Calcium, and Iron from five points on a single unprepared spinach leaf to show the variation within a single leaf:

Figure 5

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

Five points from a single fresh spinach leaf

The next table shows a five-point average from five different spinach leaves to show the variation between leaves:

Figure 6

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

Averaged values from five fresh spinach leaves

The results in Figure 5 and 6 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.

Figure 7 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.

Figure 7

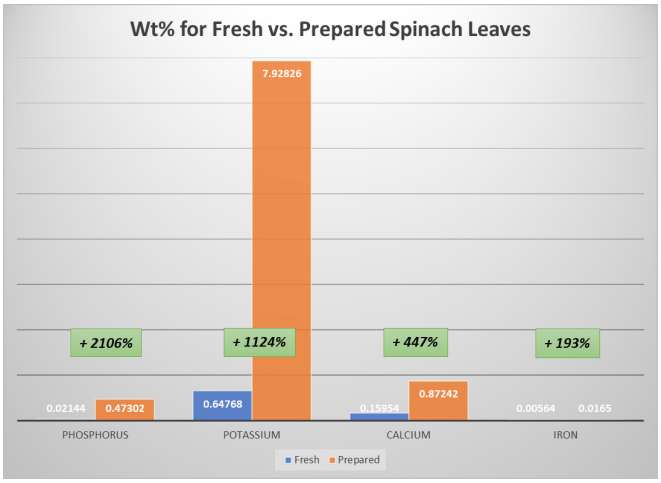
Prepared1	P	K	Ca	Fe
Pt1	0.477	7.922	0.880	0.017
Pt2	0.462	7.886	0.877	0.017
Pt3	0.483	7.998	0.877	0.016
Pt4	0.480	8.00	0.857	0.016
Pt5	0.463	7.836	0.872	0.017
Avg	0.473	7.928	0.872	0.017
StdDev	0.010	0.071	0.009	0.000
RSD%	2.088	0.898	1.055	2.308

Five points collected on the dried and prepared sample

Figure 8 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.

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 8



Fresh vs. dry nutrient concentrations, and their relative percent changes

Conclusions

The presence of approximately ninety-five percent water in the leaves has a significant impact on the results. By drying the samples, the mineral concentrations increased by many orders of magnitude. As dictated by X-ray physics, the lighter elements exhibit this to a higher degree. As the atomic number increases, the photons from that element become more energetic, and the differential between fresh versus prepared leaves decreases. In addition, by preparing the sample and homogenizing it, the stability of the results improved significantly, as shown by the RSD%.
The Bruker Plants calibration provides a versatile calibration that can be used on a variety of plant materials to attain nurtrient concentrations. However, the role of sample preparation cannot be understated, as the results can vary significantly. Measuring fresh plant material can be useful for qualitative analysis to determine if an element present or not. However, attaining the quantitative information requires the sample to be prepared, which is consistent with most operating procedures for plant analysis.

Contact Us

www.bruker.com/hhxrf

Americas / Asia / Rest of World
Kennewick, WA · USA
Tel. +1 (509) 736-2999
sales.hmp@bruker.com

Europe / Middle East / Africa
Berlin · Germany
Tel. +49 30 670990-11
sales.hmp@bruker.com