Improvement of a Microwave Digestion Method for Determination of Elements by ICP-OES in Biological Samples

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Introduction

Elemental analysis of plant material is essential to nutrient management, environmental monitoring, and heavy metals analysis.

- Reliable, well-defined analytical procedures are critical for the operation of STRAL.
- Method development is therefore necessary to optimize the elemental recovery rates in biological samples for microwave digest procedures at STRAL.
- When the NovaWAVE (SCP Science) replaced CEM's Microwave Digestion System (MDS) 2100, there was an unexpected decrease in elemental recoveries for the historic in-house plant material check (rose leaves or "RLV"). By increasing the pre-digestion time, microwave digestion time, and temperature, the Miller Digest method was optimized to restore the RLV elemental recoveries back to historic values and to improve upon those recoveries.

Photo: SCP Science

Microwave Comparison	
NovaWAVE (SCP Science)	**MDS 2100 (CEM)**
Quartz vessels	Teflon vessels
Temperature & pressure monitors for every sample	Temperature & pressure monitor for one sample only
Output temperature vs. time graphs for each sample	No graphical output
Can select different methods for individual samples	One method for entire sample tray

Table 1. A comparison of the two microwave systems.

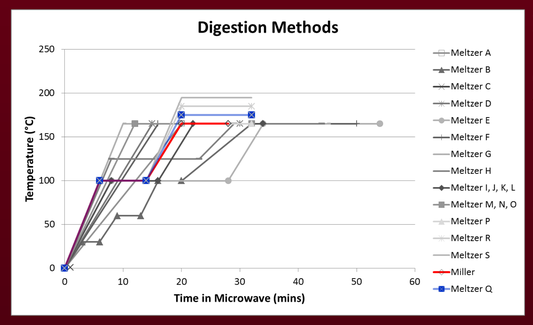


Figure 1. A graphical representation of the temperature and time steps for each method in the NovaWAVE. The Miller Digest is highlighted in red and the new method (Meltzer Q) is highlighted in blue.

Methods and Materials

- Using the historical rose leaf plant check, we compared 19 new microwave digest methods to both the historic digestion results from the old CEM MDS 2100 and the results from the new NovaWAVE.
- The NovaWAVE closed vessel system is a remarkably improved system over the CEM MDS 2100. Each sample has an individual magnetron and temperature monitor to more accurately control the digestion process for each sample.
- RLV was used because of the large amount of existing data: almost 20 years of lab-documented element concentrations. Analysis of RLV began in 1996. In total, 71 RLV samples were analyzed with a 27-element ICP method.
- The Miller Digest Method: Concentrated HNO_3 and 30% H_2O_2 digests 250 mg of ground RLV in the closed vessel NovaWAVE system.
- Method development included a pre-digestion time that varied between 0 mins, 15 mins, 30 mins, 60 mins, and 120 mins. Fig. 2.
  - There was also variation in the time between the two temperature steps (100°C, 165°C) as well as the temperature of each step. Fig. 1.
- All digestates were subsequently analyzed using ICP-OES, PerkinElmer Optima 3000.

Statistics

- An unequal variance t-test (Welch's t test) was used to compare the average elemental concentrations for each method with the historic RLV values.
  - Welch's t test: independent samples, unpaired, unequal variance, and assumptions of normal distributions with equal variances within each population.
  - Sample sizes varied. RLV samples were run in replicates of 4 for Meltzer 1 through 8. Methods A through K were run in replicates of 6.
- *Null Hypothesis* — There is no observed difference of means between the historic RLV values and the new digestion method.
- *Alternative Hypothesis* — The null hypothesis can be rejected. It can be suggested that there is an observed difference in means.

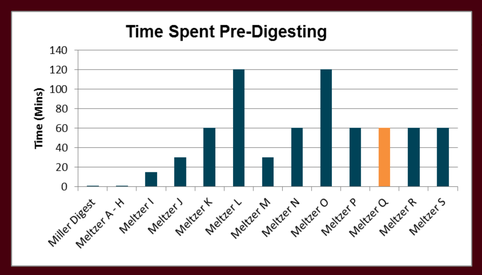
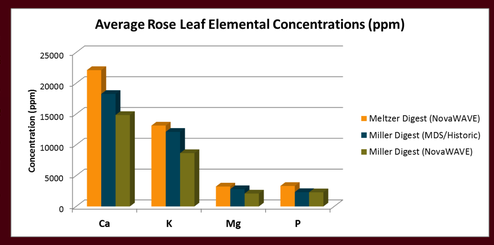


Figure 2. The amount of time spent predigesting for each method. The method highlighted in orange is the best pre-digestion time.

Figure 3. Average element concentrations for Novawave Meltzer Q, Novawave Miller Digest, and the MDS 2100 historic Miller Digest. This table shows elements with high concentrations.



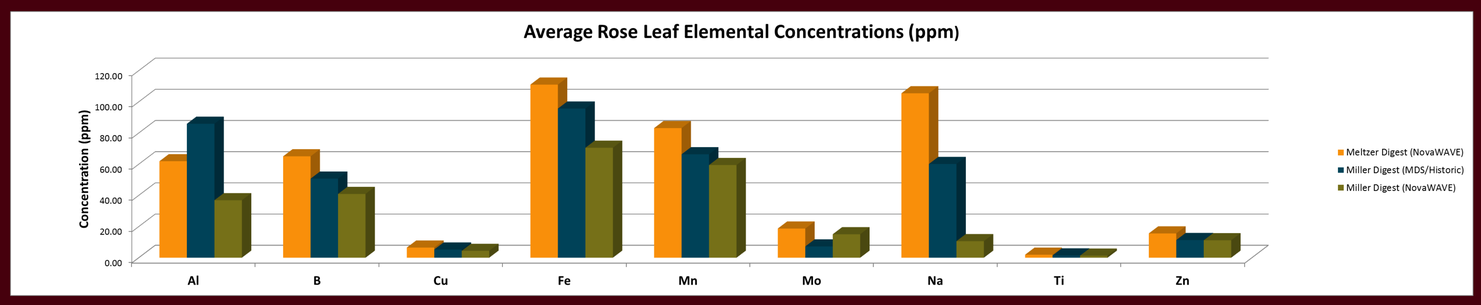


Figure 4. Average element concentrations for Novawave Meltzer Q, Novawave Miller Digest, and MDS 2100 historic Miller Digest. This table shows elements with low concentrations.

Results

- The method that returned the best statistically significant difference in means was ***Meltzer Q***.
  - ***Meltzer Q***: 60 minutes predigesting, followed by 8 mins at 100°C and 12 mins at 175°C. See Figure 1 and Figure 2.
  - Meltzer S initially chosen as the best method, however, high temperature caused ruptures of the closed vessel system and led to more errors. Lowering the temperature (from 195°C to 175°C) alleviated this problem.
- Certain elements were excluded from the statistical analysis because of a lack of data above the detection limit – Ba, Be, Li, S, Si, Ti, V.
- For **Al, As, B, Ca, Cu, Fe, K, Mg, Mo, Na, Ni, P**, and **Zn** the null hypothesis can be rejected, which suggests that the difference between the means for each method and element is **statistically significant**.

Conclusions

- Improved recoveries were observed for **B, Ca, Cu, Fe, K, Mg, Mo, Na, P,** and **Zn**.
  - Only results that rejected the null hypothesis were used in the statistical analysis.
  - The best possible (least negative for the most number of elements) difference in means, with statistical significance, was considered the best method of digestion.
  - The results from Method Q exhibited the best overall improvement in element recovery rates over the Miller Digest (NovaWAVE).
- **Al, As**, and **Ni** improved slightly over the Miller Digest (NovaWAVE) but historic averages were not achieved.
- This new method contributes to the robust method development at STRAL and has been implemented into STRAL's routine testing procedures.
- However, this research development only applies to RLV and would theoretically only apply to plant material similar to rose leaves. Further research would include an array of other plant tissues.

Sources

- *Miller RO. 1998. Chapter 8: Microwave digestion of plant material in a closed vessel. In: Handbook of reference methods of plant analysis. Kalra Y, editor. illustrated ed. CRC Press. 69 p.*