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#### **Title**

Recent developments in plant tissue analysis

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#### **Authors**

Laursen, Kristian H  
Hansen, Thomas H  
Persson, Daniel P  
et al.

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## Introduction

During the past decades several methods for plant tissue analysis have emerged, including atomic spectroscopic and spectrophotometric based analyses of plant sample digests. ICP (Inductively Coupled Plasma) based analysis is the only technique that allows multi-elemental quantification, which is very useful for both scientific and commercial purposes. Essential applications are found in *e.g.* environmental sciences, molecular biology and plant physiology. Furthermore, multi-elemental fingerprints are often used to evaluate the nutritional quality of food products or for authenticity testing – especially when combined with multivariate statistics (chemometrics). In agriculture and horticulture, plant tissue analyses are for example used to verify putative toxicities and to diagnose nutritional disorders, thereby allowing the fertilization strategy to be adjusted accordingly. Plant analyses are thus indispensable for farmers, agricultural consultants, gardeners etc. and should as often as possible complement soil analyses. Despite these obvious advantages, its dispersal is still limited in many places because the costs associated with the analyses are considered too high. However, the development of new analytical techniques might reduce expenses in the near future.

### *Full- or semi-quantitative ICP-MS analysis?*

One of the greatest breakthroughs in plant tissue analysis was the development of ICP spectrometry. This technique has now been available for more than 20 years and enables simultaneous analysis of most elements of the periodic table, even in trace concentrations. ICP spectrometry includes a wide array of analytical techniques such as ICP-Optical Emission Spectrometry (ICP-OES) and ICP-Mass Spectrometry (ICP-MS). Today, the vast amount of all multi-elemental plant tissue analyses is performed with ICP-OES. However, due to an increasing demand for low detection limits and a large linear dynamic concentration range in order to cover concentrations varying from approximately 0.01 to 15,000  $\mu\text{g g}^{-1}$  dry matter, ICP-MS is becoming the preferred analytical technique for routine analysis of plant tissues. ICP-MS enables precise and accurate detection of most elements in the periodic table in “full-quantitative” analysis mode, which relies on time-consuming acid digestion of samples and external calibration of all elements of interest. In addition, identification of spectral interferences is mandatory and methods for efficient removal of these have to be optimized. Consequently, full-quantitative ICP-MS analysis is very time and labor consuming and the multi-elemental capacity of ICP-MS is therefore rarely fully utilized. Most of the plant tissue analyses being performed today follow the full-quantitative procedure, which strongly contributes to the high analytical costs.

“Semi-quantitative” ICP-MS is a strong alternative to the full-quantitative approach and enables fast determination of the approximate elemental composition in all sample types. In semi-quantitative analysis, the mass spectrometer rapidly scans the entire mass range, thereby recording a signal for every possible element or isotope. The signals serve as input to a series of mathematical algorithms derived from a pre-calibrated internal standard containing only a few elements. As output, estimates of the concentrations of all elements in unknown samples are obtained. Thus, semi-quantitative ICP-MS relies on a very simple calibration procedure (Taylor, 2001). Analytical results obtained with semi-quantitative ICP-MS have critically been evaluated in several studies with different plant-based certified reference materials such as tomato leaves (Amarasiriwardena et al., 1990; Amarasiriwardena et al., 1997), citrus leaves (García-Alonso et al., 1997), spinach (Günther et al., 1992), sea lettuce (Hu et al., 1997), hay (García-Alonso et al., 1997) and wheat flour (Amarasiriwardena et al., 1997). These studies showed that the accuracy of semi-quantitative analysis is generally better than 70% of the true value for many elements. However, elements present in high concentrations such as Na, Mg, K and Ca are usually determined with an

inherently low accuracy when ultra-trace elements are quantified simultaneously due to the simplified calibration procedure (Amarasiriwardena et al., 1997; Entwistle and Abrahams, 1997; Soldevila et al., 1998).

In a recent study we developed a semi-quantitative ICP-MS based method for the simultaneous measurement of more than 70 elements in plant tissue. A 6-fold reduction in data acquisition time was obtained compared to full-quantitative analysis while more than three times as many elements were included. Simultaneously, an alternative high-throughput digestion method was tested, which enabled a 5-fold reduction in labor consumption for sample preparation. The developed method was applied to rice samples with the main objective to discriminate different genotypes based on multi-elemental data. Using chemometrics, it was shown for the first time that the discrimination power of semi-quantitative data was stronger than full-quantitative. Thus, the large amount of elemental information obtained with semi-quantitative ICP-MS fully outweighed the lack of accuracy compared to full-quantitative analysis (Laursen et al., 2009). Selected results from this publication are included in the results section. Furthermore, the developed semi-quantitative method was tested on 4 different reference materials to clarify the feasibility of implementing semi-quantitative ICP-MS as a routine analytical tool for plant tissue analysis.

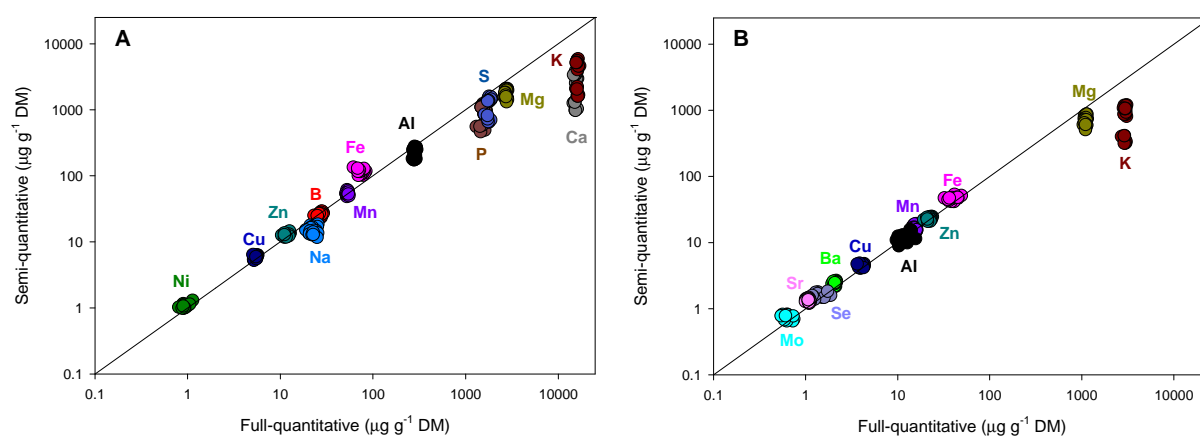
### **Materials and Methods**

All full-quantitative and semi-quantitative plant tissue analyses were performed using ICP-OES or ICP-MS, respectively. The analytical accuracy of full-quantitative versus semi-quantitative analysis was evaluated using 4 different certified reference materials (CRM), *viz.* white cabbage (BCR-679, Institute for Reference Materials and Measurements, Geel, Belgium), apple leaves and durum wheat flour (Nist 1515 and Nist 8436, respectively, National Institute of Standards and Technology, Gaithersburg, MD, USA) and leek (in-house standard, FoodDTU, Lyngby, Denmark). Subsequently, the discrimination power of full-quantitative and semi-quantitative data was evaluated with chemometrics on 24 replicates of three rice genotypes (A, B and C) differing in nutritional quality. All rice and CRM samples were initially microwave digested using a standard procedure. A high-throughput digestion method was tested on 7 samples of the durum wheat CRM and 12 samples of each rice genotype. For the semi-quantitative analysis, a  $10 \mu\text{g L}^{-1}$  multi-elemental calibration standard was used to set the response factors prior to analysis. Full-quantitative data was rejected if the accuracy was lower than 90%. In semi-quantitative analysis, the limit of detection (LOD) was generally used as inclusion criteria. LOD was determined as three times the standard deviation of minimum 7 blanks. Chemometrics was performed as principal component analysis (PCA). For further details see Laursen et al. (2009).

### **Results and discussion**

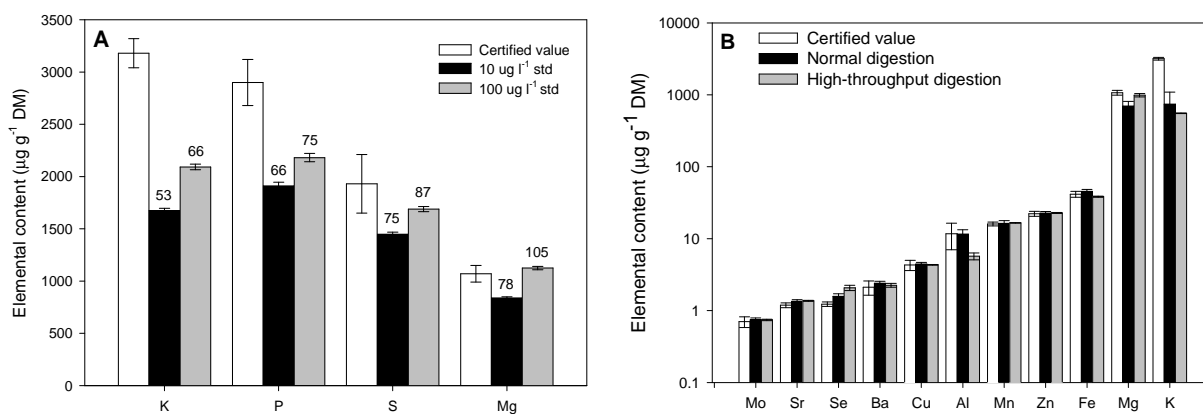
The developed semi-quantitative ICP-MS method was tested on 4 different certified reference materials and compared to full-quantitative ICP-OES results (Fig. 1). Only data for apple leaf and durum wheat are shown as results with similar accuracies was obtained for white cabbage and leek. Most elements were determined with an accuracy better than 90% in semi-quantitative analysis. However, for elements present in concentrations higher than  $1000 \mu\text{g g}^{-1}$  dry matter the accuracy decreased. This was clearly shown for *e.g.* K and Ca, confirming previous observations (Amarasiriwardena et al., 1997; Soldevila et al., 1998). The underestimation of macro-elements in semi-quantitative ICP-MS analysis is explained by the fact that a  $10 \mu\text{g l}^{-1}$  calibration standard solution was used to correct all response factors. This corresponds to a sample concentration of  $4 \mu\text{g g}^{-1}$  plant DM. The

macro-nutrients (P, Ca, Mg, S and K) are all present in concentrations exceeding 1000  $\mu\text{g g}^{-1}$  DM and the 10  $\mu\text{g l}^{-1}$  calibration standard seems to be suitable only for elements present in the range from 0.2-500  $\mu\text{g g}^{-1}$  DM. A 100  $\mu\text{g l}^{-1}$  calibration standard was therefore tested on certified durum wheat flour (Fig. 2A). It was shown that this improved the accuracy of macro-nutrients such as K, P, S and Mg significantly. For Mg, which was present in the lowest concentration of the macro-nutrients, the accuracy increased from 78 to 105%. However, a calibration standard exceeding 100  $\mu\text{g l}^{-1}$  was required to enable accurate quantification of the remaining macro-nutrients. The feasibility of using two calibration standards (10 and 1000  $\mu\text{g l}^{-1}$ ) for semi-quantitative analysis as well as inclusion of a non-equimolar calibration standard will be further investigated and presented at the conference. Simultaneous quantifications of the majority of all essential micro- and macro-nutrients is expected to be possible using these approaches without compromising the obtained reduction in data acquisition time with semi-quantitative ICP-MS.



**Fig. 1.** Full-quantitative CRM data with an accuracy better than 90% plotted against semi-quantitative data. The graphs illustrate results for apple leaf (Fig. A,  $n=20$ ) and durum wheat (Fig. B,  $n=22$ ). LOD was used as inclusion criteria for the semi-quantitative data. The axes are logarithmic and the optimal regression line through origin and with a slope of 1 is shown.

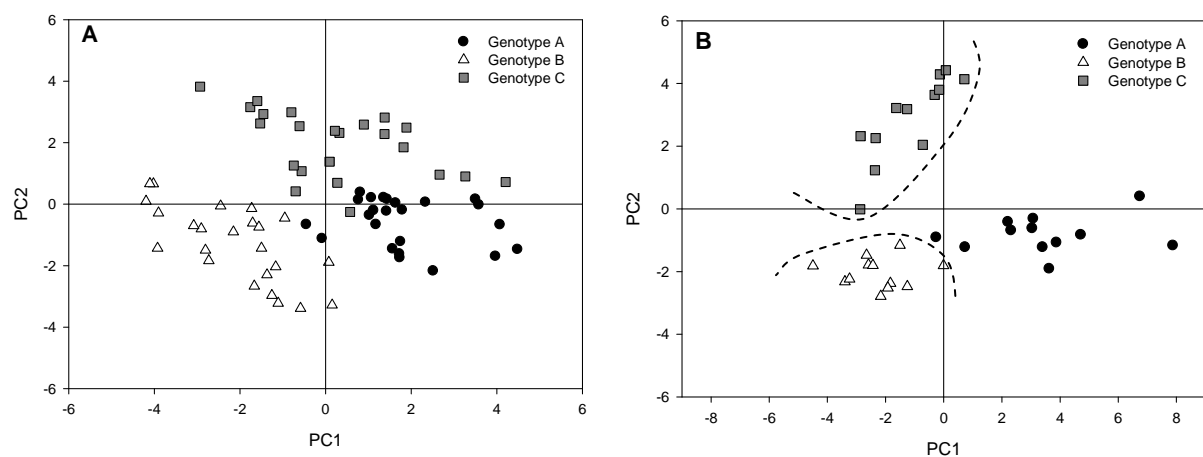
Sample digestion still represents a bottleneck in modern plant tissue analysis. A high-throughput digestion method was therefore developed and compared to a normal microwave-assisted digestion. As shown in Fig. 2B most elements in durum wheat certified reference material were determined with a high accuracy, even after high-throughput digestion (see *e.g.* Mo, Cu, Mn, Zn and Fe). Semi-quantitative ICP-MS can thus be combined with the new digestion method in order to reduce labor consumption and analytical costs. At the conference, the application of high-throughput sample digestion and semi-quantitative ICP-MS for diagnosis of nutritional disorders will be exemplified with a case study.



**Fig. 2.** Comparison of different calibration strategies (Fig. A) and evaluation of different sample digestion methods on certified durum wheat (Fig. B). In Fig. A averages  $\pm$  std ( $n=7$ ) are shown. Samples were measured semi-quantitatively with a 10 or a 100  $\mu\text{g l}^{-1}$  standard. Numbers above bars are percentage accuracy compared to the certified value. In Fig. B results from a normal ( $n=7$ ) or a high-throughput digestion method ( $n=4$ ) are shown. The y-axis in Fig. B is logarithmic.

### *Multi-elemental fingerprinting by semi-quantitative ICP-MS and chemometrics*

As a case study, three rice genotypes were analyzed with full-quantitative and semi-quantitative ICP-MS. When applying principal component analysis (PCA) to the high accuracy data (full-quantitative), discrimination of the genotypes was impossible (Fig. 3A). Using semi-quantitative analysis, a much more information rich multi-elemental fingerprint was obtained containing twice as many elements. Despite having an inherently lower accuracy, a clear discrimination of the genotypes was possible with PCA on semi-quantitative data (not shown). Even when samples from a high-throughput digestion method were analyzed with PCA, the genotypes could be discriminated (Fig. 3B). This example clearly illustrates that semi-quantitative ICP-MS in combination with chemometrics is a fast and powerful alternative to full-quantitative ICP-MS and constitutes a promising novel analytical tool for authenticity and adulteration testing of food products.



**Fig. 3.** Principal component analysis on full-quantitative (Fig. A) and semi-quantitative (Fig. B) rice data. In Fig. B, samples were digested with the high-throughput method. Fig. A is based on 15 variables (elements) above LOD with accuracy better than 90%. 32% and 20% of the dataset was explained by principal components 1 and 2, respectively. Fig. B is based on 29 elements above LOD, regardless their accuracy. 31% and 18% of the dataset was explained by principal component 1 and 2, respectively.

## Conclusion

A newly developed semi-quantitative ICP-MS method was tested as an analytical tool for plant tissue analysis. It was shown that despite relying on a simplified calibration procedure, the analytical accuracy was still acceptable for a large amount of elements when evaluated with four different certified reference materials. Even when combined with a high-throughput digestion method, essential plant micro-nutrients such as Mo, Cu, Mn, Zn and Fe were quantified accurately. It was thus shown that quantification of multi-elemental data and a significant reduction in analytical costs is indeed possible. Furthermore, a combination of chemometrics and semi-quantitative ICP-MS proved to be a promising tool for authenticity and adulteration testing. The attractiveness of plant tissue analysis is thus expected to increase by the implementation of semi-quantitative ICP-MS as a routine analytical tool, which is highly valuable for optimization of crop production in general.

## Acknowledgements

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