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Sulfur and Light Element Determination in Plant Material by X-ray Fluorescence Spectroscopy

By

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Summary

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X-ray fluorescence spectroscopy is a favourite method for the determination of light elements in organic matters. As high quality sulfur analyses have rising meaning this contribution will provide guidelines for the analytical procedure in fatty and non fatty plant materials.

Introduction

The increased interest of biological research in sulfur has raised also the demand for precise and reproducible methods of sulfur analysis. Common sulfur determination in organic matter (e.g., wet combustion and sulfur determination by ICP, gravimetry, colorimetry, turbidimetry or combustion and conductometric detection of sulfate) can hardly satisfy a high level of accuracy, precision and performance at the same time. In contrast X-ray fluorescence spectroscopy is characterized by simple sample preparation and high tolerance towards errors. Performed on multichannel spectrometers this technique provides also data of other light elements like calcium, chlorine phosphorus, potassium and silicone in the same run. This contribution will give details of sample preparation for different organic materials and will provide guidelines for calibration.

Abbreviations: GSL, glucosinolates.

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Analytical procedure for fat containing seeds

This procedure allows also the indirect determination of the total glucosinolate content in seeds of *Brassica* species and is standardised by the ISO (ISO/CD 9167.2).

Materials

Reference materials. Three suitable reference materials for *Brassica napus* with certified total S concentrations and GSL are available from the Commission of the European Communities (RM's 366, 190, 367: Commission of the European Communities - Community Bureau of Reference; BCR-XII/C/5; Rue de la Loi 200; B-1049 Brussels; Belgium; fax: +32 2 235 8072). Indicative concentrations: RM 366: 7.78 mg g⁻¹ P; 6.29 mg g⁻¹ K / RM 190: 6.68 mg g⁻¹ P; 7.54 mg g⁻¹ K / RM 367: 7.36 mg g⁻¹ P; 6.99 mg g⁻¹ K).

Synthetic samples for re-calibration covering the range of elemental concentrations are of interest. These "setup samples" should be prepared of durable materials such as glass or plastics. It is recommended to dissolve sulfates in a lithium borate glass (NORRISH & CHAPPEL 1967), or to impregnate cellulose powder (Linters, ashless quality, acid washed e.g. Macherey, Nagel & Co. MN 2200) with ammonium sulfate to compress after mixing with HOECHST wax "C" in aluminum cups under a pressure of 1 t cm⁻². Due to a higher intensity yield in the synthetic samples (SCHNUG & HANEKLAUS 1990a) final S concentrations between 1 and 7 mg S g⁻¹ cover the whole range of interest for the purpose of total S and GSL analysis in *Brassica* seeds.

Apparatus. Spoon with a capacity corresponding to approximately 20g (about 30 ml) of seeds; *Ventilated oven* maintained at 85°C or *microwave oven* (600 W) for drying seeds if necessary; *Blender* (coffee grinder type): 100 cm² volume, 8 cm diameter, 180 W power; *Spatula*; *liquid cuvettes* (e.g. CHEMPLEX 1540) covered with *mylar film* 6 µm gauge (e.g. CHEMPLEX 250); *hand-press* allowing (repeatable) application of a defined pressure; *high dispersive X-ray spectrometer* (dispersion better than 50 eV) with vacuum equipment (vacuum better than 1 torr is sufficient (SCHNUG & HANEKLAUS 1990b) or helium purge with proposed settings: Rh-tube (40 kV, 4.5 mA), Ge-crystal, Ar-CH₄ flow counter, He purge, 100 seconds counting time).

Method

Sample preparation. If the moisture of the seed sample exceeds 10 %, it must be reduced to 7-9 % (this moisture content corresponds to the term "air-dry") by drying about 40 g (60 ml) of the seeds spread out in a thin layer either in a ventilated oven for 75 minutes at 85 °C or in a microwave oven for 2 minutes at 600 W (SCHNUG & HANEKLAUS 1990b). Allow samples to cool to room temperature before proceeding. Spoon about 20 g (30 ml) of air dry seed into the blender and grind for 30 seconds. Scrape off any meal adhering to the wall of the blender by means of the spatula and grind the sample again for **not more than** one second,

to ensure homogenization. Fill a liquid cuvette with homogenized meal to the upper edge. Handle the cuvettes gently after filling to avoid separation of particles of different size. Compress the meal by means of a hand-press under a pressure that reduces the volume of the meal to 35 % of the original volume. In either case, the pressure applied to all samples, including the reference samples, should be the same within ± 10 % deviation. The amount of ground material used in the aluminum cups or liquid cuvettes depends only on their size and does not influence the determination itself. These amounts however should be kept constant for one laboratory and apparatus. A detailed description of errors caused by different sources of variations in any step of the preparation procedure for the X-RF method is given by SCHNUG & HANEKLAUS 1990b.

The poor penetration of light element $K\alpha$ radiation (RABE 1975) allows the preparation of small sample sizes down to 50 seeds (250 mg) by grinding samples **and standards** (!) by means of a common pepper grinder and compressing thin layers into liquid cuvettes (SCHNUG & al. 1992). For the analysis of single seeds the seed is glued with acetylated glucose into a hemispherical hole prepared in the centre of a wax-pellet and sectioned horizontally (SCHNUG & al. 1992).

Calibration for seeds. Due to the high oil content it is impossible to grind whole seeds of *Brassica* to the particle size which is usually required for the X-RF analysis of light elements. As the intensity peak depends upon the particle size distribution in the ground seed sample (SCHNUG & HANEKLAUS 1990b) it is necessary to standardise all steps of the sample preparation within one laboratory. To overcome the natural inhomogeneity of rapeseed samples (SCHNUG & HANEKLAUS 1990b) measurements of the intensity of the $K\alpha$ radiation of the individual elements must be made on three separate sub-samples of each rapeseed reference material. For other elements than sulfur this calibration has to be transferred to physically durable synthetic samples. It should be mentioned that differences in the intensity yield influence the element concentrations calculated for the synthetic samples after calibrating with seed standards and do not therefore represent the true element concentrations in the synthetic material. These calculated values for the synthetic samples are only valid for measurement of seed samples from which the original calibration has been derived. During routine usage the calibration of the spectrometer has to be verified twice a day by use of the synthetic standards. A check by use of whole seed standard reference material is recommended on a monthly basis. Linear regression equations derived from given concentrations and collected net $K\alpha$ intensities for the standard samples are used for the calculation of element concentrations in unknown samples. Corrections for matrix effects and interelement excitation are not necessary (SCHNUG & HANEKLAUS 1990b).

Analytical procedure for non fatty organic materials (<2 % fat)

Materials

Reference materials. National Bureau of Standards (NBS) Washington "Citrus Leaves" NBS 1572.

Synthetic samples for re-calibration have to be prepared by spiking samples of already ground organic material by wetting the samples with solutions made of ammonia or nitrate salts of the elements resulting in final element concentrations of 0.5-10 mg g⁻¹ P; 0.5-15 mg g⁻¹ S; 5-50 mg g⁻¹ K; 3-30 mg g⁻¹ Ca; 0.1-1 mg g⁻¹ Cl and 1-100 mg g⁻¹ Si (for this element pure SiO₄ must be used). Homogenization and grinding after drying to less than 1 % moisture are carried out as described below.

Binder. HOECHST wax type "C".

Apparatus. To grind hard and/or fiber containing materials to a particle size less than 100 µm additionally to the equipment necessary for the application of X-RF spectroscopy to seeds (listed above) a suitable grinder is needed (e.g. RETSCH ZM1 Ultracentrifugal mill).

Method

Sample preparation. Grind the dried sample (moisture content below 1 %) in an ultracentrifugal mill to a particle size less than 100 µm. For low fiber containing materials, like young leaves of *Brassica* species, suitable fineness can be obtained by grinding the **hot** sample in a coffee grinder type blender (see above). Mix 1.1 g of the dry and finely ground standard and sample material with exactly 4.4 g wax. Fill the homogeneous mixture into an aluminum cup and compact by means of a hydraulic press at a pressure of 1t cm⁻².

Calibration for non-fatty organic materials. Linear regression equations derived from given concentrations and collected net K α intensities for the standard samples and a blank (pure compressed wax) are used for the calculation of element concentrations in unknown samples. Due to the dilution of the material corrections for matrix effects and interelement excitation are not necessary (RABE 1975).

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