

A Rapid Method for total glucosinolate determination in oilseed rape seeds by means of low resolution energy dispersive X-ray fluorescence spectroscopy

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INTRODUCTION

The indirect determination of the total glucosinolate content of rapeseed via the analysis of total sulphur in the seeds by X-ray fluorescence spectroscopy ('X-RF method') is a well proved method which has been standardised by the ISO (1991) and adopted as official method by the Commission of the European Communities (SCHNUG and HANEKLAUS, 1991). The only disadvantage of the X-RF method are the high investment costs which are typical for wavelength dispersive X-RF spectrometers.

Beside the need for highly accurate glucosinolate analysis for official certification and research, separation of seed batches is a field of application which fits well to the fast performance of the method but not to the costs of the instruments. Due to lower requirements in accuracy this task might be fulfilled by simpler constructed and thus much cheaper energy dispersive X-RF spectrometers with low resolution proportional counters and excitation by nuclide sources. Aim of the research work reported here was to investigate the possibilities of low resolution energy dispersive X-RF spectrometers for glucosinolate analysis and to evaluate statistical quality parameters for the data obtained by this machines.

MATERIAL and METHODS

Origin of samples: calibration standards: Community Bureau of Reference (BCR) Brussels oilseed rape standard reference materials Nr. RM 366, RM 190 and RM 367 (WAGSTAFFE et al., 1992).

Check samples: 82 winter oilseed rape samples ex harvest 1990 from field experiments and field surveys of the Department for Agriculture of the University of Newcastle upon Tyne.

Sample preparation: following the proposed ISO procedure described by SCHNUG and HANEKLAUS (1990) which may be modified for small sample sizes (SCHNUG et al., 1993).

X-ray fluorescence spectrometers used: PHILIPS PW 1410 wavelength dispersive X-RF (Cr-tube 60 kV 30 mA, PE crystal, vacuum path, Ar-CH₄-flow counter, measuring time: 20 seconds); AUTOKUMPU X-MET 820 energy dispersive X-RF (1.48 GBq Fe-55 source; element channels counted: S, P, Cl, K, Ca and backscatter, each with settings as provided by the instrument supplier; measuring time: 240 seconds)

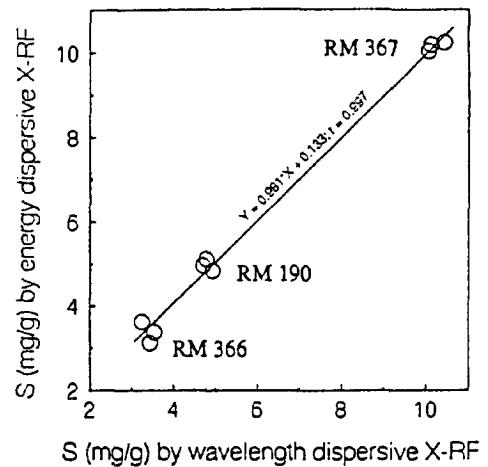
Calibration procedures: calibration for sulphur based on three replicates of the BCR standard reference materials (SCHNUG et al., 1992). On the wavelength dispersive X-RF only the intensities of the S-K α radiation, on the energy dispersive X-RF also the K α intensities for P, Cl, K, Ca and the intensity of the backscatter were collected.

For the energy dispersive instrument the best fitting correction system (lowest value for the residuals) has been used for further measurements. The calibration procedure for the wavelength dispersive instrument and the algorithms for the conversion of the total sulphur concentrations into total glucosinolate concentrations are described in detail by SCHNUG et al. (1992).

RESULTS AND DISCUSSION

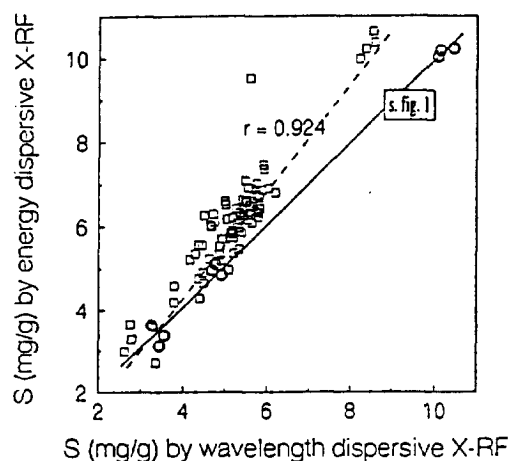
Calibration for total sulphur with BCR RM's : Figure 1 displays the excellent results of a comparison of total sulphur concentrations obtained from both instrument types following a calibration with the three BCR rapeseed reference materials.

Fig. 1 : Sulphur concentrations determined in BCR reference rapeseed materials RM 366, RM 190 and RM 367 measured by means of wavelength- and low resolution energy- dispersive X-ray fluorescence spectroscopy.



Validation of the calibration for total sulphur for the energy dispersive X-RF: Although there is still a very good correlation between the data for unknown samples analyzed with both instrument types ($r = 0.924$ for data marked with squares in fig. 2) the data do not fit to what was measured in the BCR standard materials (fig 1, and data marked with circles in fig. 2). It is obvious from the results presented in figure 2 that the application of this calibration to the unknown samples gives systematically misleading results. The relation between the sulphur concentration and the recorded S-K α intensity is not linear over the whole concentration range provided by the BCR standards and that the settings of the instrument are only suitable for sulphur concentrations lower than 0.7% (which refers to the highest sulphur concentration in the NIAB sample set used for the X-MET application reported by NUOTIO and HOLTARI (1991)). It is quite obvious from the results presented in figure 2 that the counter runs into the Geiger range (HAHN-WEINHEIMER et al., 1984) if sulphur concentrations are higher than 0.45% and that concentrations above 0.7% S cause an overflow of the proportional counter.

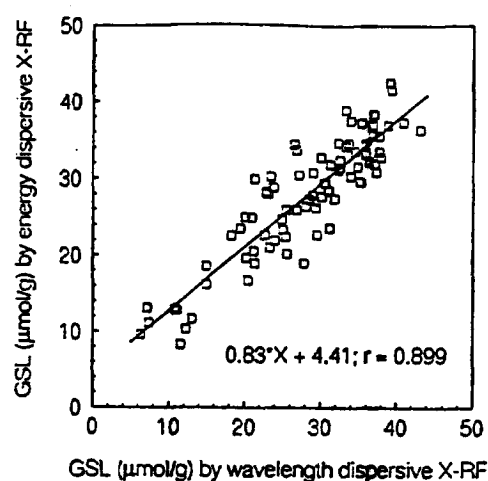
Fig. 2 : Sulphur concentrations determined in rapeseeds measured by means of wavelength- and low resolution energy- dispersive X-ray fluorescence spectroscopy.



To solve this problem and in order to investigate the suitability of energy dispersive X-RF for total glucosinolate analysis all samples with sulphur concentrations above 0.7% has been excluded and after that the total sulphur concentrations obtained from the energy dispersive X-RF (X) were fitted to the values of the wavelength dispersive system (Y) by a linear transformation: $Y = 0.686 \cdot X + 1.055$.

Figure 3 displays the results for the fitted data after calculation of the total glucosinolate content from the total sulphur concentrations. The standard error of estimation for that regression is calculated to $3.5 \mu\text{mol/g}$ which is two times higher than those obtained from wavelength dispersive instruments ($1.3 \mu\text{mol/g}$) but still much lower than those derived from routine NIR instruments with fixed filters ($8.9 - 18.9 \mu\text{mol/g}$) (RENARD et al., 1987; SCHNUG and HANEKLAUS, 1991; STARR et al., 1985).

Fig. 3: Total glucosinolate concentrations in rapeseeds measured by means of wavelength- and low resolution energy- dispersive X-ray fluorescence spectroscopy.



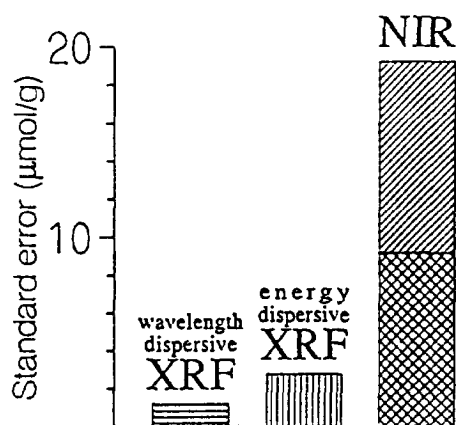
CONCLUSIONS

From the results of the investigations done on the application of energy dispersive X-RF instruments for total glucosinolate analysis in oilseed rape seeds the following conclusions can be drawn:

Due to the high standard of reproducibility and repeatability which is already provided by wavelength X-RF machines it is hardly to expect to see this type of energy dispersive X-RF instrument entering the field of analyses for official certifications or research purposes.

However, small energy dispersive X-RF instruments seem to be suitable for the separation of yield batches according to their glucosinolate content. If there is no need to cover the whole range of glucosinolate concentrations in rapeseeds ($< 5 - > 120 \mu\text{mol/g}$) there is a good chance to enhance the accuracy of energy dispersive machines by optimizing the settings of the proportional counter for a smaller range of sulphur concentrations. This, however, requires that the user need to be supplied with suitable set of rapeseed reference materials in the interesting range, which is different from what is actually offered by the BCR.

Although this investigation reveal that energy dispersive X-RF spectrometers might be an interesting alternative to NIR machines, which are in a similar cost range but with a definitively lower accuracy for the determination of total glucosinolates (fig. 4). Although the accuracy and the statistics for the results obtained from energy dispersive X-RF are much better than those derived from NIR it should be kept in mind, that NIR provides more analytical possibilities for an agrochemical laboratory (e.g. protein and oil determination; KOESTER, 1989).



ABSTRACT

Due to the high standard of reproducibility and repeatability which is already provided by wavelength X-RF spectrometers it's difficult to see low resolution energy dispersive X-RF instruments entering the field of analyses for official certifications or research purposes. This type of X-RF machines, however, seems to be suitable for the separation of yield batches according to their glucosinolate content. The standard error of estimation for that application is calculated to 3.5 µmol/g which is two times higher than those obtained from wavelength dispersive instruments (1.3 µmol/g) but, however, still much lower than those derived from routine NIR instruments with fixed filters (8.9 - 18.9 µmol/g). Thus this instrument might be an interesting alternative to NIR machines, which are in the same cost range. Although the statistical values of the results obtained from energy dispersive X-RF are much better than those derived from NIR it should be kept in mind, that NIR provides more analytical possibilities for an agrochemical laboratory (e.g. protein and oil determination). For the separation of seed batches it's not necessary to cover the range of high glucosinolate concentrations so that the settings of the instrument can be optimized to the lower range of concentrations. This, however, requires that the user need to be supplied with an adapted set of rapeseed reference materials for the calibration in a certain range.

Zusammenfassung: Eine Schnellmethode zur Bestimmung des Gesamtglucosinolatgehaltes von Rapssaat mittels niedrigauflösender energiedispersiver Röntgenfluoreszenzspektroskopie.

Der hohe Standard wellenlängendispersiver Röntgenfluoreszenzspektrometer für die Reproduzierbarkeit und Vergleichbarkeit von Meßergebnissen läßt nicht erwarten, daß niedrigauflösende energiedispersive Varianten dieser Instrumente im Bereich von Forschung und Attestierung zum Einsatz kommen werden. Mit einem mittleren Standardfehler von 3.5 µmol/g, welcher zwar doppelt so hoch liegt als bei Verwendung wellenlängendispersiver Instrumente (1.3 µmol/g), aber immer noch weit unter dem von Filter-NIR Geräten (8.9 - 18.9 µmol/g), erscheinen diese Geräte jedoch für die Separierung von Partien gut geeignet.

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