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Experiences with the X-RF Method for the Determination of Total Glucosinolate Content in Rapeseed

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INTRODUCTION

The change of rapeseed cropping to varieties with low glucosinolate content requires analytical methods which are fast, precise and reliable. None of the methods commonly used (chromatography, enzymatic release, colorimetry, near-infrared-refelectance) is able to realize all three features at the same time. The latest development in glucosinolate analysis is the "X-RF" method according to SCHNUG and HANEKLAUS (1), which is at this time the most favourable technique within the EEC. For the last two years it is estimated that more than 90% of all analyses for intervention purposes has been carried out by the X-RF method. Aims of this contribution are a brief explanation of principles and new developments of this method.

PRINCIPLES

The X-RF method is based on two principles: the close relationship between total sulphur and total glucosinolate content in rapeseed and the distinctive applicabilities of X-ray fluorescence spectroscopy for total sulphur determination in organic matter.

The reason for the close relationship between total sulphur and total glucosinolate content is due to the fact that more than 99% of the sulphur in rapeseed is bound in proteins and glucosinolates whereof the sulphur in the protein fraction is a farely constant factor (tab.1).

Tab. 1: Characteristic concentrations and variabilities of sulphur fractions in rapeseeds and calculated maximum error for total glucosinolate determinations by the X-RF method

source of variation	typical concentrations in rapeseeds (µg/g)		maximum error * (µmol/g)
	average	standard-deviation	
PROTEIN-SULPHUR			
aminoacid pattern			
- cystein	1780	28	0.4
- methionin	1153	32	0.5
total protein concentration	2933	200	3.1
GLUCOSINOLATE SULPHUR			
- 0-varieties	6440	4508	
- 00-varieties	1288	759	
SULPHATE-SULPHUR	30	17	0.3
OTHERS **	6	2	<0.1

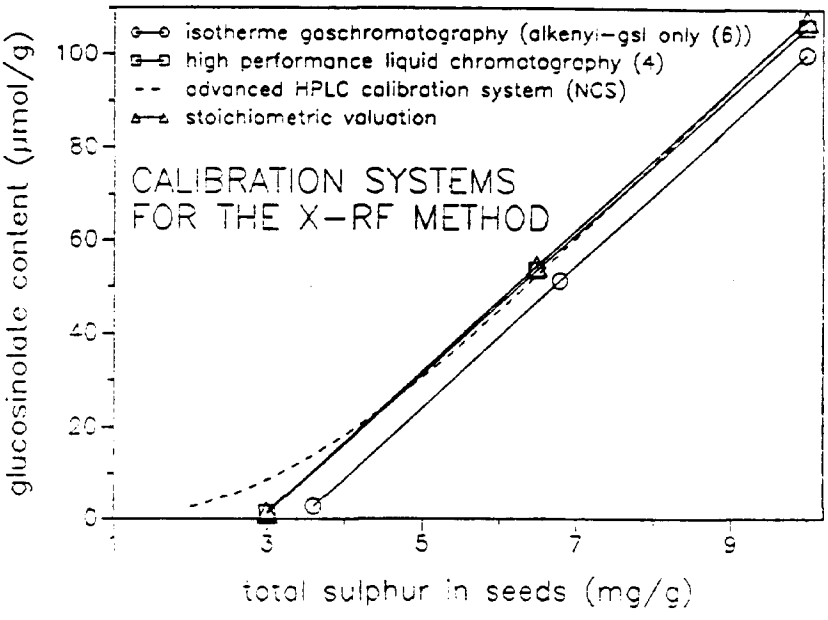
[* for the estimation of total glucosinolate concentration from total sulphur concentration
** eg. sulfolipids, APS/PAPS, glutathione]

Therefore variations in the total sulphur content in rapeseed are almost exclusively caused by different glucosinolate concentrations due to genetical (2) and environmental (3,8) factors. According to literature and experimental data the (theoretical) maximum error by calculating the glucosinolate content from total sulphur concentration caused by natural variations in the amino-acid composition is supposed to be lower than 0.5 µmol/g; according to experience changes in total protein content of seeds may result in maximum errors of less than 2 µmol/g for each percent protein deviation below 19 or above 25 % protein.

STANDARDIZATION

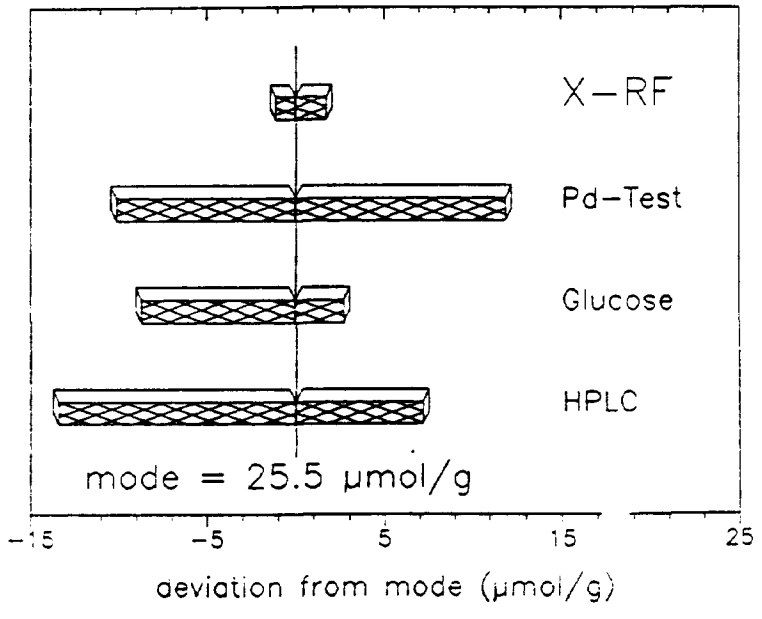
By use of the X-RF method results for total glucosinolate content can be derived from stoichiometric calculations or according to calibrations against a certain reference method (fig. 1). At this time a linear calibration system based on HPLC analysis of 4 alkenyl- and 2 indolyl- glucosinolates (according to EEC directions) is used for certification of seeds.

Fig. 1: Comparison of calibration functions for the X-RF method



The reliability of this system has been proved in several national and international ring tests (fig.2); typical statistical data derived from wavelength dispersive spectrometers are 1.9 µmol/g for repeatability and 3.8 µmol/g for reproducibility (4). On average the results of the HPLC calibration system are only 0.5 µmol/g lower than stoichiometric values, which take all existing glucosinolates into account. Therefore stoichiometric valuation and HPLC-calibration enables an independent mutual confirmation.

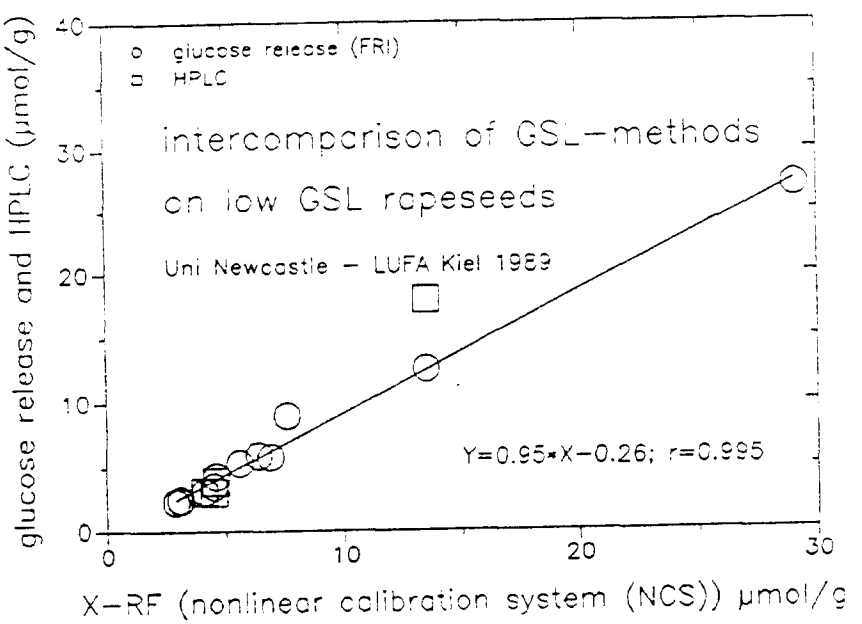
Fig. 2: Variation of results for the total glucosinolate content in a rapeseed sample derived from different analytical methods - BCR ring test 1988 (7) (BCR CRM 190 - mean: 24.6 µmol/g; mode of 165 measurements from 16 laboratories with 7 methods = 25.5 µmol/g



However, few practical experiences have shown that sometimes in the low range of double lows (<10 µmol/g) the linear HPLC calibration system tends to too low values. This problem is now solved by a non linear calibration system (fig. 1). The results of one of the latest intercomparisons of the X-RF method with nonlinear calibration system (NCS) with glucose release and HPLC in the range of low glucosinolate content in rapeseed is presented in figure 3.

Modifications of all calibration functions presented in figure 1 are now also available for the quality control of rapeseed meal (5). The idea for the application of the X-RF method to rapeseed meal is the reidentification of the quality of the rapeseed originally used for crushing. Thus together with a fast method for this type of samples also a new evaluation system for rapeseed meal is introduced (5).

Fig. 3: Results of an intercomparison of methods for glucosinolate determination in the range of low glucosinolate content in rapeseed (9).



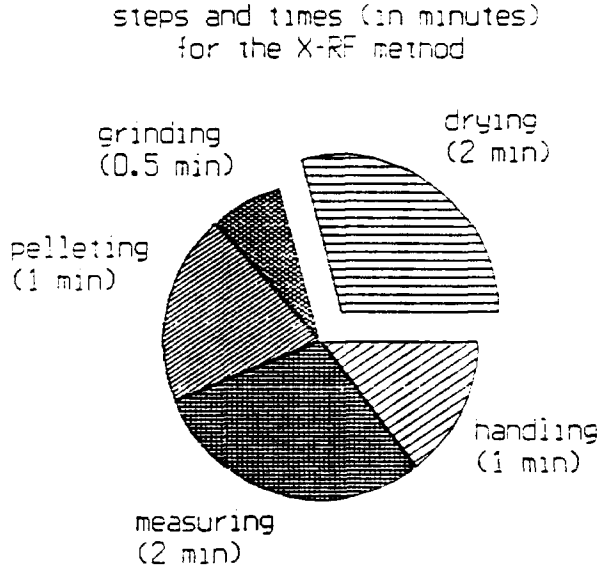
PREPARATION TECHNIQUES

Several effective improvements in preparation techniques for the X-RF method for seed and meal analyses (5) has been developed. By use disposable polyethylene sample cups covered with polyethylene film and a specific pressing equipment two persons are able to prepare up to 100 samples/h for measurement. Usually the whole procedure for a single sample needs less than 5 minutes per sample (fig. 4). Pellets treated this way are now also usable in

vacuum spectrometers which helps also to reduce the variable costs for gas in helium purged instruments.

Analysis of single seeds is possible by use of an imbed technique based on wax and carboxilated glucose. For this purposes by use of a small heated steel ball a hole of little more than half the diameter depth than a single rapeseed is melted in the center of a pellet prepared by pressing of HOECHST wax "C" in which the seed is glued. The glue is prepared by boiling of glucose with acetic acid. After hardening of the glue the seed is divided by use of a razor blade giving an equal surface to the wax pellet.

Fig. 4: Steps and times for the X-RF method



FUTURE ASPECTS

The excellent results of the X-RF method in recent ring tests can be accounted to the unique calibration standards and algorithms of all participants. In order to keep this high standard in glucosinolate analysis organization of calibration of the X-RF method will be done under supervision of the Community Bureau for Reference (BCR) in Brussels. The first set of standards released by the BCR especially designed for the calibration of the X-RF method will be available bevore harvest campaign 1989 (9).

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ANNEX

Adress of the BCR: Community Bureau of Reference (BCR) - Rue de la Loi - B-1049 Brussels (Belgium) - Telex: COMEU B 21877 - Fax: 32 2 236 33 07

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Total Glucosinolate Content in Rapeseed Using Reflectance

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Abstract

A method has been developed for the determination of total glucosinolate content in rapeseed. Following selective hydrolysis of glucosinolates by the endogeneous myrosinase in rapeseed, the glucose produced is detected with commercially available glucose test strips. The intensity of the colour on the strips is measured using a portable reflectometer. This method is suitable for use by plant breeders and for the determination of glucosinolate content in commercial rapeseed loads.

Introduction

Several methods are available for the quantitative determination of glucosinolates in rapeseed. The High Pressure Liquid Chromatography (HPLC) method for separating desulfoglucosinolates can provide a measure of the content of individual glucosinolates including indole glucosinolates (1) . This method, however, is not the method of choice for most routine plant breeding purposes where a total glucosinolate figure is all that is required.

A number of methods are available for the estimation of total glucosinolate content in rapeseed for example, thymol (2), chloropalladate (3) and techniques based on glucose release (4). Some of the advantages and disadvantages of these methods are discussed at the end of this paper. The aim of this project was to develop a method for measuring total glucosinolate content which was rapid, simple, lacked any chromatographic steps and which did not require the use of expensive equipment.

Experimental Determination of Glucosinolate Content in Rapeseed.

1. Weigh 200 mg air-dried rapeseed into a 10 ml centrifuge tube.
2. Add 3.0 ml of 50 mM Glycine-NaOH buffer (pH 9.0) and homogenise thoroughly (15 seconds). Rinse the ultra turrax shaft with 2x1.0 ml aliquots of buffer solution dispensed through the top hole in the shaft.
3. Mix the tube gently and leave for 10 minutes.
4. Add 1.0 ml of chloroform, seal the tube and mix thoroughly by shaking.
5. Add 50ul 10% chlorohexidine diacetate in methanol and mix.
6. Add 1.0 ml of 100 mM Citric Acid/Sodium Citrate buffer pH 5.0