Institute of Plant Nutrition and Soil Science

Elke Mareke Bloem
Silvia Haneklaus
Ewald Schnug

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Relation between total sulphur analysed by ICP-AES and glucosinolates in oilseed rape and Indian Mustard seeds

Elke Bloem, Silvia Haneklaus and Ewald Schnug[1]

Abstract

With the introduction of double low oilseed rape in the beginning of the 90´s a fast and reliable method for the determination of glucosinolates became important in order to separate different qualities of seeds of oilseed rape. At that time an indirect method for the determination of glucosinolates was developed by Schnug & Haneklaus (1987a, c) based on the close relationship between total sulphur determined by X-RF and glucosinolates in seeds of oilseed rape. The method has been extremely successful, but is still expensive in terms of investment.

It was the aim of this contribution to test if total sulphur determinations by wet digestion and ICP-AES which is available in a wide range of laboratories is also a suitable method for the indirect determination of glucosinolates. The results of the ICP-AES measurement were in good agreement with the X-RF method and the same equation could be used for the indirect calculation of glucosinolates in seeds of oilseed rape.

The indirect determination of glucosinolates which was originally developed for oilseed rape was also tested for Indian Mustard. Indian Mustard too revealed a close relationship between total sulphur and glucosinolates. This is a prerequisite for the use of the indirect method but the strongly changing protein contents and seed weights made the development of special algorithms for Indian Mustard necessary.

Keywords: glucosinolates, indirect determination, ICP-AES, sulphur, x-ray fluorescence spectroscopy

Zusammenfassung

Beziehung zwischen dem mit ICP-AES gemessenen Gesamtschwefelgehalt und dem Glucosinolatgehalt in Samen von Raps und Senf

Mit Einführung der glucosinolatarmen Winterrapssorten zu Beginn der 90´er Jahre wurde eine schnelle und sichere Bestimmungsmethode für den Glucosinolatgehalt von Rapsproben notwendig, um schnell und verlässlich unterschiedliche Qualitäten beim Raps unterscheiden zu können. Zu dieser Zeit entwickelten Schnug & Haneklaus (1987a, c) ein indirektes Verfahren zur Bestimmung des Gesamtglucosinolatgehaltes in Rapssaaten. Dieses basierte auf der engen Beziehung, die zwischen dem Gesamtglucosinolatgehalt und dem mit RFA ermittelten Gesamtschwefelgehalt im Samen von Raps besteht. Es war das Ziel dieses Beitrages zu testen, inwieweit die Gesamtschwefelanalyse mittels Mikrowelle mit nachfolgender Bestimmung mit ICP-AES geeignet ist, den Glucosinolatgehalt von Raps indirekt zu bestimmen.

Die Ergebnisse der ICP-AES-Analyse stimmten derart gut mit den Werten, die mit RFA bestimmt wurden, überein, dass sogar der gleiche Algorithmus für die indirekte Glucosinolatbestimmung mit ICP-AES verwendet werden konnte.

Des weiteren wurde untersucht, ob sich die indirekte Glucosinolatbestimmung, die für Winterraps getestet und entwickelt wurde, auch auf eine andere Ölfrucht (*Brassica juncea*) übertragen lässt.

Schlüsselworte: Glucosinolate, indirekte Bestimmung, ICP-AES, Schwefel, Röntgenfluoreszenzspektroskopie

[1] Institute of Plant Nutrition and Soil Science, Federal Agricultural Research Centre (FAL), Bundesallee 50, 38116 Braunschweig/Germany; e-mail: elke.bloem@fal.de

1 Introduction

Oilseed rape (*Brassica napus* L.) is the most important oilseed crop in Europe while in India the Indian Mustard (*Brassica juncea* L.) is the second most important source of edible oil after groundnut (Singh et al., 2000). Production of oilseed rape aims to produce seeds which are rich in oil and low in glucosinolates while an important criteria in mustard production is a high glucosinolate content. Oilseed rape is used for cooking oil, is a component of different foodstuffs and is used as a raw material for different industrial products (Toepfer et al., 1995; Bona et al., 1999). Additionally the rapeseed meal is a valuable product for animal nutrition since the introduction of oilseed rape varieties which are low in erucic acid (<2 %) and glucosinolates because of the raw protein content being higher than 30 % and a well-balanced amino acid composition (Kaminska et al., 2000; Kokkonen et al., 2000; Mullan et al., 2000).

The change in rapeseed cropping to varieties with low glucosinolate contents in the beginning of the 90´s raised the need for analytical methods which were fast, precise and reliable in the determination of glucosinolates. Only rapeseed meal with a glucosinolates content lower than 20 μmol g^{-1} met the desired standard quality for which a subsidy was paid (Schnug & Haneklaus, 1990a). None of the methods which were commonly used at that time (chromatography, enzymatic release, colourimetry, near-infrared-reflectance) were able to realise all three features at the same time (Schnug et al, 1990). Therefore Schnug and Haneklaus (1987a, 1987c, 1988) developed an indirect method for the determination of glucosinolates based on total sulphur (S) determination employing X-ray fluorescence spectroscopy (X-RF). This is up to now the most favourable technique for a fast and precise determination of total glucosinolates in seeds and besides the determination by HPLC the only method approved by the European Union (EU) and ISO (ISO/CD (1991) 9167.2).

The X-RF method is based on two principles: the close relationship between total S and total glucosinolate content in rapeseed and the distinctive applicability of X-ray fluorescence spectroscopy for total S determination in organic matter (Schnug et al. 1990). The whole procedure consists of only three simple steps, grinding of the seeds in a common coffee mill, pelleting in a hydraulic press and measuring of the S-K_α radiation in an X-ray spectrometer taking not more than 5 minutes for one sample (Schnug & Haneklaus, 1987a, b).

The close relationship between total S and total glucosinolates content results from the fact that more than 99 % of the S in rapeseed is bound in proteins and glucosinolates whereof the S in the protein fraction of seeds is a fairly constant factor (Schnug et al. 1990). In contrast with vegetative plant parts the sulphate concentration in seeds is very low (Schnug & Haneklaus, 1987b). Therefore variations in the total S content in rapeseeds are almost exclusively caused by different glucosinolate concentrations due to genetical and environmental factors (Finlayson, 1977; Rakow, 1983; Schnug, 1989). According to literature and experimental data Schnug et al. (1990) calculated a maximum error of lower than 0.5 μmol g^{-1} by calculating the glucosinolates content from total S concentration caused by natural variation in the amino acid composition.

The following non-linear algorithm describes the relationship between total S and total glucosinolates in seeds of oilseed rape (Schnug et al., 1990; Schnug & Haneklaus, 1990a):

$$GSL = -0.12 \cdot S^3 + 2.8 \cdot S^2 - 5.6 \cdot S + 3.5 \qquad (1)$$

GSL = total glucosinolate content [μmol g^{-1}]
S = total sulphur content [mg g^{-1}]

The commonly used methods for the determination of glucosinolates (GC, HPLC) are still labour-intensive and time consuming. Therefore indirect methods like the X-RF method are up to now recommendable alternatives.

Since the beginning of the 90´s S deficiency is a widespread problem in European agriculture and therefore S analysis in plant materials are often routinely conducted. Therefore it is of interest to know if other methods for the determination of total S can be used instead of X-RF for the indirect determination of glucosinolates. However, the accuracy of the indirect method depends mainly on the reliability of the S determination. The most important difference of the X-RF method compared to other methods for S determination is that no initial destruction of organic matter by digestion with acids or ashing of the sample is necessary, through which losses of S are always possible. Crosland et al. (2001) showed in an inter-laboratory comparison of ten laboratories where total S was determined by ICP, turbidimetric methods, precipitation with barium chloride followed by analysis by Atomic Absorption Spectroscopy and Dumas method that the coefficient of variation (CV) for plant total S analysis ranged from 8.2 to 20.3 %. Such a great variation makes it impossible to use other methods of total S analysis for the indirect determination of glucosinolates without prior comparison of the methods. The aim of this work was to evaluate the suitability of total S analysis by microwave digestion followed by ICP-AES analysis for the indirect determination of total glucosinolates in oilseed rape because ICP-AES is available in a wide range of laboratories in contrast to X-RF.

Additionally, it was investigated if the given algorithm can also be used for other oil crops like Indian Mustard. For India such a fast method for the determination of glucosinolates would be of particular significance as the glucosinolate content is the most important quality criteria of Indian Mustard.

2 Materials and Methods

2.1 Origin of the samples:

Different samples were compared in this methodological investigation:

1. Samples of winter oilseed rape (*Brassica napus* L.) (n = 576) from two different varieties with low glucosinolate content grown over 3 years (2001-2003) at three different sites (two sites in Scotland one site in Germany) with and without S application (data from Salac, 2005);
2. Samples of winter oilseed rape (*Brassica napus* L.) (n = 18) with high glucosinolate contents grown in Braunschweig (2000-2001) with and without S application;
3. Samples of Indian Mustard (*Brassica juncea*) (n = 25) grown commercially in India and representing a wide spectra of different qualities.

2.2 Analysis:

S_t: The total S content in seeds was determined by microwave digestion: ground seed material was digested for 20 minutes in a microwave oven using a mixture of HNO_3 (65 %) and H_2O_2 (35 %) (4:1, vv). After cooling, samples were diluted with bi-distilled water to a final volume of 25 ml, filtered and measured by inductively coupled plasma-atomic emission spectroscopy (ICP-AES).

N_t: The total nitrogen content in seeds was determined by using *Kjeldahl* determination.

GSL: Glucosinolates in seeds were determined according to the EU method L170/28 (Anon, 1990). The desulphoglucosinolates were measured by HPLC with UV detection at 229 nm using acetonitrile (20 %) and water as eluents and a LiChroCart 250-RP18 column for seperation of the glucosinolates.

Protein: N_t * 6.25.

Oil content: Soxhlet solvent extraction method.

Weight per volume: 20 ml of the seeds were weight.

3 Results and Discussion

3.1 Relation between total S analysed by ICP-AES and glucosinolates in oilseed rape

Up to the time when the X-RF method was developed by Schnug and Haneklaus (1987c), there were generally only poor methods available for the determination of total S which were either time consuming or low in accuracy. The determination of total S in plant materials was not routinely carried out because the problem of S deficiency was just arising at the end of the 80´s and S was no yield limiting factor in the past. Today the S determination in plant materials is part of the routine analysis, and other recommendable methods beside the X-RF method are available, such as microwave digestion followed up by measurement with ICP-AES. It was the aim of this study to evaluate if this method is also appropriate to determine the glucosinolates content in rapeseeds indirectly.

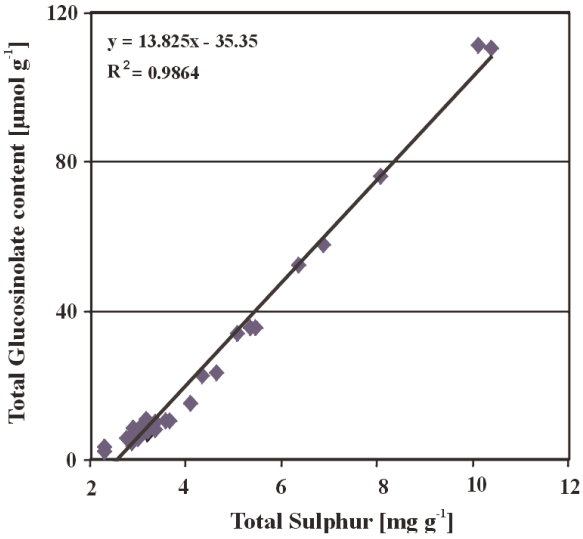

Figure 1:
Relationship between total glucosinolate content measured by HPLC and total sulphur determined by ICP-AES in seeds of oilseed rape (*Brassica napus* L.)

The strong relationship between the total glucosinolate and the S content in rapeseeds could also be proven when the samples were digested by microwave and measured by ICP-AES (Figure 1).

The algorithm which describes the relationship between glucosinolates and total S which was developed by Schnug et al. (1990) can also be used the other way round to calculate the S content in seeds of oilseed rape using the glucosinolate content.

In figure 2 the theoretical S content in seeds of oilseed rape was calculated in this way via the glucosinolate content. The calculated X-RF values were compared with the measurements from ICP-AES (figure 2a). The difference between both values was extremely low. On average the values of the ICP-AES measurement were around 0.28 mg S g^{-1} higher than the calculated X-RF values. The results of both methods of S determination showed a close correlation of 99 % (figure 2b).

The results clearly demonstrate that microwave digestion of oilseed rape samples in combination with the measurement of S by ICP-AES is a sufficiently accurate method which can be used for the indirect determination of glucosinolates. ICP-AES delivers values which are in good agreement with X-RF measurements. It is possible to use the same equation for the indirect determination of glucosinolates which is shown in figure 3, where the measured values are plotted against the equation which was found by Schnug & Haneklaus (1990a) by using X-RF.

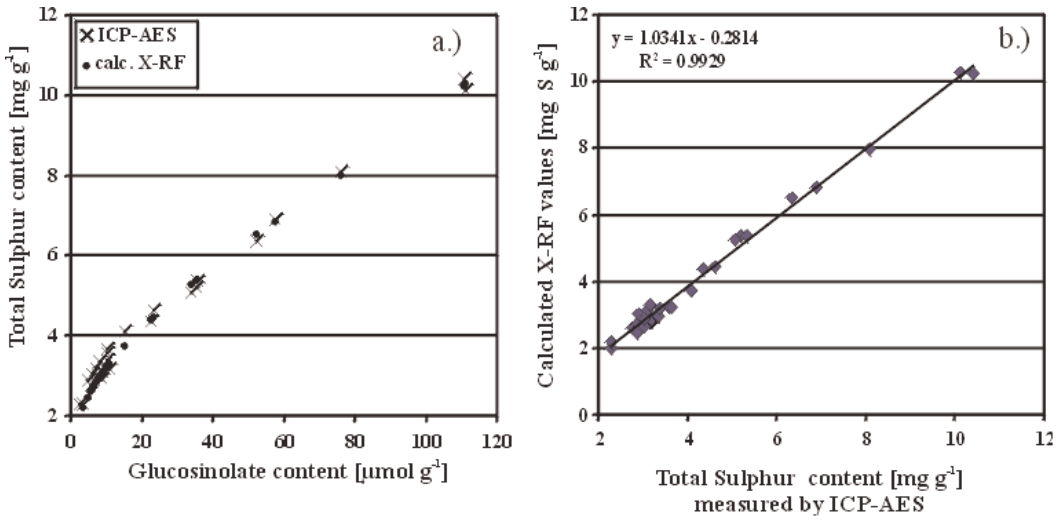

Figure 2a:
Comparison of the total S content measured by ICP-AES and calculated from the glucosinolates content
Figure 2b:
Relationship between the total S content measured by ICP-AES and the X-RF values calculated via the glucosinolate content

Figure 3 also demonstrates the extremely high stability of the indirect glucosinolate determination because the samples which are shown in figure 3 were from different varieties (Lipton, Bristol, Jet Neuf, Ceres), different sites (Braunschweig, Inverness and Aberdeen), different years (2000-2003) and differed in their S nutritional status from S deficient to optimum S supply. All these factors did not affect the reliability of the indirect glucosinolate determination through total S determination.

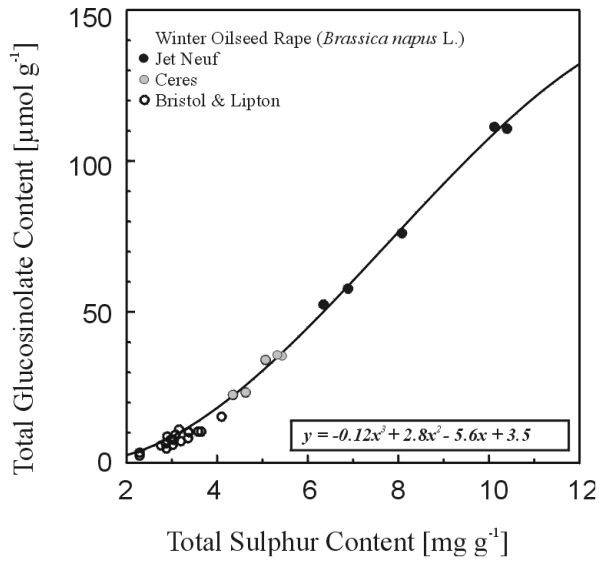

Figure 3:
Relationship between glucosinolates and total sulphur determined by ICP-AES in seeds of oilseed rape in comparison with the equation found by Schnug & Haneklaus (1990a) using X-RF

3.2 Applicability of the method for the indirect determination of glucosinolates via total sulphur to Indian Mustard (Brassica juncea L.)

Indian Mustard also revealed a close relationship between glucosinolates and total S but the measured values differed from the equation obtained by Schnug & Haneklaus (1990a) (Figure 4). The seeds from Indian Mustard differed widely in some important features (Table 1) which were affecting the indirect determination of glucosinolates, such as protein content and weight per volume.

The calibration function for the X-RF method is valid for rapeseeds with a concentration in a "normal" range of 19-23 % crude protein, 39 - 45 % oil and 6-9 % moisture. In cases of extreme deviations in the oil or protein content of the seeds a systematic error in the results obtained with

Table 1:
Variation of different characters of Indian Mustard

	Total S mg g^{-1}	Total GSL µmol g^{-1}	Total N mg g^{-1}	Protein content %	Oil content %	1000-seed weight g	Weight per volume mg ml^{-1}
Minimum	4.4	12.8	24.89	15.56	36.56	1.31	236
Maximum	10.3	131.8	41.31	25.82	39.11	4.63	691

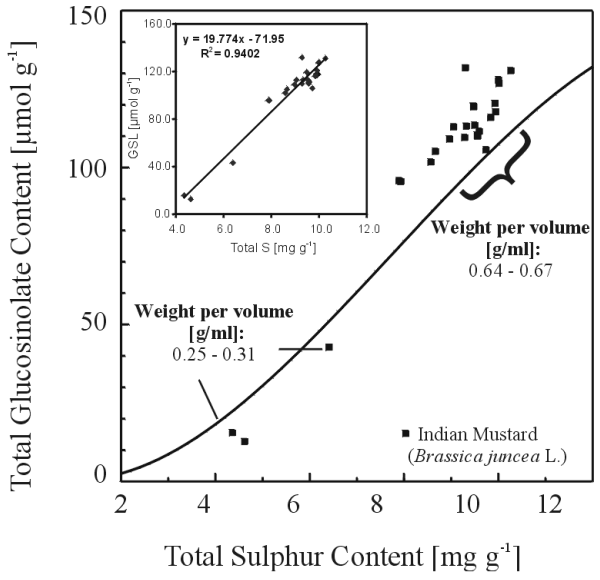

Figure 4:
Relationship between glucosinolates and total sulphur determined by ICP-AES in seeds of Indian Mustard in comparison with the equation found by Schnug & Haneklaus (1990a) using X-RF for oilseed rape

the X-RF method may occur (Schnug & Haneklaus, 1990b). This was obviously the case for the Indian Mustard samples as there were some samples with a very low weight per thousand seeds, where the glucosinolate content was lower than it could be expected from the total S content (figure 4). The Indian Mustard seeds had a much higher variability in seed sizes, which obviously affected the calculation of glucosinolates from the S content. One reason among others might be that with the smaller sample size used for the chemical S determination, inhomogenities in the sample become more important. The same problem may also have resulted in less accurate N concentrations so that a stoechiometric calculation could not be applied.

A higher protein content is generally a sign of a lower glucosinolate content in oilseed rape and vice versa. In case of Indian Mustard, the samples with the lowest S contents had also the lowest glucosinolate, nitrogen and therefore protein contents. Additionally, these samples were extremely light with a low weight per thousand seeds and also a low weight per volume. In contrast, oilseed rape shows an extremely constant weight per thousand seeds and under insufficient nutritional conditions rape plants produce a lower number, but intact seeds (Schnug & Haneklaus, 1994, Schnug & Haneklaus, 1998). Therefore size and weight of the seeds of oilseed rape are not affected by both nutritional or environmental factors.

Because of these differences it was not possible to use the procedures proposed by Schnug and Haneklaus (1990b) to compensate the error in the glucosinolate content which is caused by the changing protein content.

With multiple regression it was tested which of the analysed parameters were the best to improve the calculation of glucosinolates in seeds from total S determination. The S content alone could explain the glucosinolate content only by around 94 % (figure 4). The second most important parameter was the weight per volume. Considering both factors the glucosinolate content could be explained to 97% by the following algorithm:

$$GSL = 12.57 \cdot S + 4.66 \cdot V - 65.34 \quad (2)$$

GSL = total glucosinolate content [µmol g^{-1}]
S = total sulphur content [mg g^{-1}]
V = Volume weight [g 20 ml^{-1}]

Protein and oil content as well as 1000-seed weight yielded no significant contribution to the multiple regression. The differences among the seed samples, however, were well described by their volume weight, which when considered in a multiple regression equation successfully corrected the GSL data.

4 Conclusion

In the beginning of the 1990´s when the indirect method for the determination of glucosinolates in oilseed rape was developed, most methods for the determination of total S were both time consuming and low in accuracy. Therefore the X-RF determination of total S was the only method which was used for the indirect determination of glucosinolates. Today methods like microwave digestion followed by ICP-AES measurement are of high accuracy and the results of this work show that this method can also be used for the indirect determination of glucosinolates in seeds of oilseed rape. The values correspond very well with those measured by X-RF. ICP-AES is a widespread analytical equipment in many laboratories while X-RF is still an expensive equipment. Therefore it is a great advantage that the indirect glucosinolate analysis can also be carried out in labs which are equipped with ICP-AES. However, it is necessary to mention here, that this indirect method can not be used for official measurements because this is restricted to S determination by X-RF.

A special task of this research work was to check the applicability of the basic calibration equation for other *Brassica* seeds than *Brassica napus*. The accuracy of S determination is a prerequisite for the method and requires well-proven standards of the same matrix. The results show that *Brassica juncea* differs in some important features from *Brassica napus* and therefore the algorithm used for *Brassica napus* is not directly applicable to *Brassica juncea*. The data needed to be corrected by the volume weight which was a strongly variable factor in *Brassica juncea*.

Therefore, it is necessary to test for each crop if the indirect method is directly applicable or if an additional correction is required for the investigated crop.

References

Anon (1990) Bestimmung des Glucosinolatgehaltes von Oelsaaten durch HPLC. EU Methode Nr. L170/28, 1990

Bona S, Mosca G, Vamerali T (1999) Oil crops for biodiesel production in Italy. Renewable Energy 16(1-4):1053-1056

Crosland AR, Zhao FJ, McGrath S (2001) Inter-laboratory comparison of sulphur and nitrogen analysis in plants and soils. Commun Soil Sci Plant Anal 32(5&6):685-695

Finlayson AJ (1977) The chemistry of the constituents of rapeseed meal. Publication / Rapeseed Assoc of Canada 45:124-136

ISO/CD (1991) 9167.2 - Rapeseed : determination of total glucosinolates ; part 2: X-Ray Fluorescence Spectrometry (XRF)

Kaminska BZ, Brzoska F, Skraba B (2000) High-protein fraction of 00 type rapeseed meal in broiler nutrition. J Anim Feed Sci 9(1):123-136

Kokkonen T, Tuori M, Leivonen V, Syrjala-Qvist L (2000) Effect of silage dry matter content and rapeseed meal supplementation on dairy cows. 1. Milk production and feed utilisation. Anim Feed Sci Technol 84(3-4):213-228

Mullan BP, Pluske JR, Allen J, Harris DJ (2000) Evaluation of Western Australian canola meal for growing pigs. Aust J Agric Res 51(5):547-553

Rakow G (1983) Zur Variabilität von Öl- und Proteingehalten in Zuchtstämmen von Winterraps (*Brassica napus* L.) In: Actes du congrès tome 1 ; 6e Congrès International sur le Colza, Paris, 17-18-19 Mai 1983, Paris, pp 115-118

Salac I (2005) Influence of the sulphur and nitrogen supply on S metabolites involved in Sulphur Induced Resistance (SIR) of *Brassica napus* L.. Braunschweig : FAL, 134, 85 p, Landbauforsch Völkenrode SH 277

Schnug E (1988) Quantitative und qualitative Aspekte der Diagnose und Therapie der Schwefelversorgung von Raps (*Brassica napus* L.) unter besonderer Berücksichtigung glucosinolatarmer Sorten. 256 p, Kiel, Univ, Habil-Schr, 1988

Schnug E (1989) Double low oilseed rape in West Germany – sulphur nutrition and glucosinolates levels. Asp Appl Biol 23:67-82

Schnug E, Haneklaus S (1987a) Schnelle und präzise Bestimmung des Gesamtglucosinolatgehaltes von Rapssamen – Vergleich der Röntgenfluoreszenzanalyse mit Gaschromatographie und Hochdruckflüssigkeitschromatographie. Fette, Seifen, Anstrichmittel 89(1):32-36

Schnug E, Haneklaus S (1987b) X-ray fluorescence-spectroscopy - a new method for precise, rapid and simple determination of total glucosinolate content of rapeseed. Proc. 7th Int. Rapeseed Congr., Poznan (Poland) 1528-1534

Schnug E, Haneklaus S (1987c) Indirekte Bestimmung des Gesamtglucosinolatgehaltes von Rapssamen mittels Röntgenfluoreszenzanalyse. Fresenius Z Anal Chem 326(5):441-445

Schnug E, Haneklaus S (1988) Theoretical principles for the indirect determination of the total glucosinolate content in rapeseed and meal quantifying the sulphur concentration via X-RF fluorescence (X-RF method). J Sci Food Agric 45:243-254

Schnug E, Haneklaus S (1990a) A rapid method for the evaluation of the glucosinolate quality of extracted rapeseed meal by total sulphur determination via X-ray fluorescence spectroscopy. Fett Wiss Techn 92(2):57-61

Schnug E, Haneklaus S (1990b) A systematical study on factors influencing the determination of the total glucosinolates content in rapeseed by the X-RF method. Fett Wiss Techn 92(3):101-106

Schnug E, Haneklaus S, Wathelet JP (1990) Experiences with the X-RF method for the determination of the total glucosinolate content in rapeseed. GCIRC-Bulletin (6):120-125

Schnug E, Haneklaus S (1994) Sulphur deficiency in Brassica napus : biochemistry – symptomatology, morphogenesis. Braunschweig : FAL, 31 p, Landbauforsch Völkenrode SH 144

Schnug E, Haneklaus S (1998) Diagnosis of sulphur nutrition. Nutr Ecosystems 2:1-38

Singh B, Kumar A, Yadav YP, Dhankhar RS (2000) Response of Brassicas to sulphur application. Indian J Agron 45 (4):752-755

Toepfer R, Martini N, Schell J (1995) Modification of plant lipid synthesis. Science 268:681-686