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Determination of total and destroyed glucosinolates in processed oilseed rape meal

SILVIA HANEKLAUS und EWALD SCHNUG

Institute of Plant Nutrition and Soil Science

Introduction

The striking problem of the analysis of GSL in meals is the fact that approximately 50 % of the GSL, which are originally in the seeds, are decomposed during processing and crushing (Daun, 1986), yielding a wide spectrum of breakdown products (Gardrat and Prevot, 1987). In a model experiment Buchner (1988) detected beside intact GSL, 18 different decomposition products in hydrolysed rapeseed meal from which only 12 could be identified. In addition to this, the toxicological importance of individual intact GSL and their corresponding breakdown products is not fully clear. Thus it seems to be fairly difficult to evaluate the hazardous potential of rapeseed meals by its individual components. It's easy too, to understand that such a complex system may cause certain difficulties for chromatographic methods.

The proposed new way for the evaluation of the GSL quality of the meal is based on the idea of a close relationship between the total S content in seeds and corresponding meals after crushing. One example supporting this relationship is the fact that only very low amounts of S are lost during crushing to extract the oil (Abraham and Man, 1987) and that the loss of volatile compounds can be evaluated from the share of steam volatile GSL in the seeds. Thus it should be possible to calculate the total S content and in that way also the total GSL content of the seeds originally used for crushing from the total S content of the meal.

This principle of a "re-identification" of the quality of the original seed from which the meal has been produced has two main advantages: First, that the total S content in organic materials can be analysed easily, rapidly and precisely by use of X-ray fluorescence spectroscopy; twice, the possibility to determine the meal quality (which currently has no legal definition) by seed quality, which is exactly defined by law. So a "double low rapeseed meal" could legally be defined as a meal, which is definitively produced by the crushing of a double low rapeseed. Analytical details for this procedure are described below in part "A": "reidentification of the GSL-content of the original seed".

The extension of the X-RF method to processed meals is the logical consequence of the idea that glucosinolates are important antinutritive compounds in animal diets. The proposed principle of "re-identification" assumes a linear dependency between the total GSL content of a certain seed batch and the utilization of the meal for foodstuff production, but gives no direct information about the toxicity of the meal itself. One possible way to increase the amount of lack of information seems to be the evaluation of the amount of GSL destroyed during processing. This parameter can be calculated from the amount of free sulphate in the meal, because sulphate is absent in oilseed rape seeds and only released during decomposition by biochemical or physicochemical processes (Schnug, 1987; Aitzetmueller, 1988). In order to avoid enzymatic destruction of GSL during the analysis an extraction with 1m ascorbic acid is recommended. For further analytical details one should refer to Schnug (1987).

By subtraction of the amounts of destroyed GSL also informations about intact GSL in the meal will be enabled. Common sulphate determination by chemical or chromatographic methods, however, requires skilled staff, special equipment and last but not least simply time. Thus in part "B" of this draft a procedure for sulphate determination in rapeseed meal is described, which can easily and quickly be carried out on X-RF spectrometers. This procedure involve only three mayor steps: 1: chemical inactivation of residual myrosinase activity, precipitation of sulphate as $BaSO_4$; 2: filtration or centrifugation; 3: determination of residual Ba concentration in the filtrate (or in the supernatant solution after centrifugation) by X-RF spectroscopy. For further details of Ba determination in solutions one may refer to Visapää (1963).

Part "A": Reidentification of the GSL-content of the original seed

Materials

Calibrants

Reference material with certified sulphur content. National Bureau of Standards (NBS) Washington "Citrus Leaves" NBS 1572.

Synthetic standards. A set of synthetic standards has to be prepared by spiking samples of double low meal with S by wetting the samples with ammoniumsulphate solution resulting in final S concentrations of 3-12 mg/g S. Homogenisation and grinding after drying to less than 2 % moisture are carried out as described below.

Synthetic samples for re-calibration covering the range of S content are of interest. These "setup samples" should be prepared of durable materials such as glass or plastics. It is recommended to dissolve sulphates in a lithium borate glass (Norrish and Chappel, 1967), or to impregnate cellulose powder (Linters, ashless quality, acid washed e.g. Macherey, Nagel & Co. MN 2200) with ammonium sulphate to compress after mixing with HOECHST wax "C" in aluminium cups under a pressure of 1t/cm2. Due to a higher intensity yield in the synthetic samples (Schnug and Haneklaus, 1990a) final S concentrations between 3 and 12 mg S/g cover the whole range of interest for the purpose of total GSL analysis in processed meals.

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 mill type): 100 cm^2 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 and Haneklaus, 1990b) or helium purge, settings according to manufacturers recommendations typical example for OXFORD-QX: Rh-target (40 kV, 4.5 mA), Ge-crystal, Ar-CH_4 flow counter, He purge, 100 seconds counting time),
- Glass plate and a glass powder-funnel of 20 cm and 15 cm diameter respectively.

Method

Sample preparation

Spoon about 20 g (30 ml) of the meal onto the glass plate, spread it out in a thin layer, cover it with the glass powder-funnel (which acts as condenser for the moisture in the sample) and heat it for 60 seconds in a microwave oven.

Transfer the hot sample immediately (do not allow to cool!) to the blender and grind it 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 homogenisation.

Fill a liquid cuvette with homogenised 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 aluminium 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 comprehensive description of the evaluation of details of the method is given by Schnug and Haneklaus (1990c).

Calibration for processed meal

As shown by Schnug and Haneklaus (1990c) a linear relation exists between the S concentrations and the S-Kα intensity in meal samples prepared as described above. The total sulphur in the sample is derived by comparing the intensity of the S-Kα radiation with those derived from synthetic standards and the reference material.

To recalculate the total GSL content of the original seed from the total S-content of the extracted meal three principle conversions of the total S-content derived from the X-ray analysis are necessary: Conversions for changes in oil and moisture content, a consideration of S-losses during processing and finally the calculation of the total GSL content in the seeds by the corrected S-content of the meal.

During processing of rapeseeds changes in oil and water content take place which cause a concentration of S in the meal. The first step in calculating the S content of the original seed (S_{seed}) from the S content in the meal (S_{seed}) is therefore the correction for changes in water and oil concentrations by use of a concentration factor (F; default value = 1,12) in the algorithm:

$$S_{seed} = S_{seed}/F$$

in which F derives from the formula:

$$F = 100/(100-((O_{seed}-O_{meal})+(M_{seed}-M_{meal}))$$

O_{seed}= oil content in seeds (default value = 42 %); O_{meal}= oil content in the meal already prepared for analysis (default value = 3 %); M_{seed}= moisture content in seeds (default value = 8 %); M_{meal}= moisture content in the meal already prepared for analysis (default value = 2 %)

Sulphur losses with the extracted oil are extremely low (Abraham and Man, 1987) and need therefore not to be considered in the calculations. The alkenyl GSL gluconapin and glucobrassicanapin,however, are steam volatile compounds and therefore may be completely lost during processing. The seed content of steam volatile GSL is in close relation to the total GSL content of the seeds the loss of S bound to those compounds during processing is at least a function of the total glucosinolate and thus the S content of the original seed (Schnug and Haneklaus, 1990c).

By the following algorithm the S content of the original seed (S_{seed}, including S in the steam volatile GSL) from the total S content in the meal (S_{seed}) can be delivered:

$$S_{seed} = ((S_{meal}-1.845)/(0.708/(2/F)))/F$$

Below S concentrations of 6.3 mg/g in the meal corrections for steam volatile GSL seem to be not necessary (Schnug and Haneklaus, 1990c).

For the final calculation of the GSL content by the S concentrations in the original seed the following formulas have to be used:

for S concentrations below 4.93 mg/g :

$$GSL\ (\mu mol/g) = 2.53*S + 0.768*S^2 - 5.035$$

for S concentrations above 4.93 mg/g :

$$GSL\ (\mu mol/g) = 14.233*S - 43.815$$

Part "B": Determination of destroyed glucosinolates in processed oilseed rape meals

Materials

Chemicals

Extraction solution (5 mmol Ba & 1 m Ascorbic acid prepared from dissolving 1.2215g $BaCl_2x2H_2O$ and 176.1g ascorbic acid in deionised water to 1000 ml. This solution enables to cover a range of up to 50µmol/g destroyed GSL in meals.

Barium standard solutions prepared by dilution of the extraction solution (described above) with 1m ascorbic acid.

Apparatus

- Polypropylene beakers 250ml volume,
- Laboratory balance with 0.1g accuracy (for preparation of stock solutions use an analytical balance with appropriate accuracy!),
- Homogenisator e.g. Ultrathurax,
- Filter funnels and filter paper suitable for Ba-sulphate filtration (e.g. Schleicher and Schuell "Blauband") or Laboratory centrifuge with suitable vials (e.g. 15 ml volume, sharp bottom),
- Transferpipette 10ml volume,
- Liquid cuvettes (e.g. CHEMPLEX 1530) covered with mylar film 6 µm gauge (e.g. CHEMPLEX 250),
- High dispersive X-ray spectrometer (dispersion better than 50 eV) running on normal atmospheric path; suitable combinations of excitation source, crystal and counter may vary between different spectrometer types and should be discussed with the manufacturer in order to provide maximum intensity yields for the Ba fluorescence radiation. For a PHILIPS PW 1410 spectrometer the following settings are proposed: Mo-target (60 kV, 50 mA), LIF220-crystal (focused on Ba-Lα, Ar-CH_4 flow counter + scintillation counter, 150 seconds counting time; this configuration yield approximately 4 counts sec^{-1} μmol^{-1}.

Method

Sample extraction

Weight 10g of the rapeseed meal in a 250ml PP-beaker, add 100ml of Ba/ascorbic acid extraction solution and homogenise by means of the Ultrathurax equipment.

Allow 30 minutes for precipitation of bariumsulphate before further processing.

Separate solution and solids by filtration through a suitable filter paper. Be patient this procedure is painfully slow! In cases were a higher number of samples need to be processed centrifugation of the decanted supernatant solution (approx. 10 minutes at 2000 rpm) is recommended. If in the filtrate or the centrifugated solution still precipitation of bariumsulphate takes place the time for extraction/reaction needs to be increased or the separation process need to be carried out twice.

Barium determination via x-rf

Transfer 10ml of the standard- and sample solutions into a liquid cuvette and count the intensity of the Ba-Lα radiation and the background in a distance of ±2.5° from the Ba-La peak.

As shown by Visapää (1963) a linear relation exists between the Ba concentrations and the Ba-Kα intensity in meal samples prepared as described above. The total sulphur in the sample is derived by comparing the intensity of the Ba-Kα radiation with those derived from standard solutions.

Results

The amount of Ba in µmol determined in the sample solution is equivalent the amount of sulphate in the processed rapeseed meal and thus equivalent the amount of destroyed glucosinolates (GSL destroyed in meal). The results should be expressed on a dry matter basis.

Considering the amount of GSL reidentified in the original seed (see part "A" above), the amount of intact glucosinolates in the processed meal is derived from:

$GSL_{intact\ in\ meal} = GSL_{reidentified\ in\ original\ seed} * F - GSL_{destroyed\ in\ meal}$

The percentage of glucosinolates destroyed during processing is calculated by:

$GSL_{destroyed\ in\ meal} / (GSL_{reidentified\ in\ original\ seed} * F) * 100.$

Bestimmung des Gesamtglucosinolategehaltes und deren Abbauprodukte in Rapsschrot

Die GSL Analyse in Rapsschrot wird im Vergleich zur Saat erheblich durch die Vielzahl der während der Verarbeitung entstehenden Abbauprodukte erschwert. Darüber hinaus liegen nur wenige Anhaltspunkte bezüglich der Toxikologie einzelner GSL vor. Um dennoch die Qualität von Rapsschroten zu bestimmen, wurde ein neuer Ansatz realisiert, welcher auf der engen Beziehung zwischen dem Gesamtschwelfgehalt von Saat und Schrot beruht. Die RFA-Methode zur Bestimmung des GSL-Gehaltes in Rapssaaten könnte dadurch bei Schroten zur Reidentifizierung der Qualitäten der eingesetzten Saaten angewandt werden. Um einen enzymatischen Abbau der GSL während der Analyse der Proben zu vermeiden, wird eine Extraktion in 1m Ascorbinsäure empfohlen. Die Bestimmung des Anteils von GSL-Abbauprodukten, die durch die Verarbeitung entstehen, kann über die Bestimmung der Sulfatkonzentrationen ermittelt werden, da in intakten Rapssamen kein Sulfat nachweisbar ist.

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Verfasser: Haneklaus, Sivlia, Dr. sc. agr.; Schnug, Ewald, Dr. sc. agr. Dr. habil., Institut für Pflanzenernährung und Bodenkunde der Bundesforschungsanstalt für Landwirtschaft Braunschweig-Völkenrode (FAL), Leiter: Dir. und Prof. Dr. Dr. Ewald Schnug.