OIL CROPS: BRASSICA SUBNETWORK

PROCEEDINGS OF THE THIRD WORKSHOP, QUALITY TRAINING, AND CHINESE PROJECT REPORTS, HELD IN SHANGHAI, PEOPLE'S REPUBLIC OF CHINA, 21–24 APRIL 1990

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Oil Crops: Brassica Subnetwork

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TABLE OF CONTENTS

PART III
BRASSICA SUB-NETWORK COUNTRY PRESENTATIONS

PART IV
BRASSICA SUB-NETWORK:
DISCUSSIONS / RECOMMENDATION

- - - - - xxx - - - - -

DETERMINATION OF GLUCOSINOLATE CONTENT BY GAS CHROMATOGRAPHY OF TRIMETHYLSILYL DERIVATIVES OF GLUCOSE

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1. APPLICATION

The method is intended for determining the glucosinolate composition and content (1 to 200 μmoles per gram oil-extracted meal) of seed or meal samples of rapeseed/canola (*Brassica campestris* L. and *B. napus* L.). It may also be applied to the analysis of seed or meal samples of brown or Oriental (yellow seeded) mustard, *B. juncea* (L.). Coss., and white mustard, *Sinapsis alba* L.

2. PRINCIPLE

Endogenous enzymes, including myrosinase, are inactivated and glucosinolates extracted using boiling water. The glucosinolates are purified on an ion-exchange column then hydrolyzed to release glucose with the addition of myrosinase to the column. The glucose released is derivatized to form volatile trimethylsilyl (TMS) ethers which are separated and quantified, using myo-inositol as internal standard, by gas liquid chromatography.

3. BACKGROUND

Gas chromatography of trimethylsilyl derivatives of desulfated glucosinolates is one of the most useful approaches to date for determining the glucosinolate content of rapeseed (McGregor et al. 1983). In addition to determining glucosinolate content it also provides information on glucosinolate composition as almost all of the glucosinolates known to be present in rapeseed are well resolved. However, as individual glucosinolates are measured separately it suffers from possible accumulative errors resulting from the summing of the individual peaks.

Gas chromatography of trimethylsilyl derivatives of myrosinase released glucose avoids this problem. The method was originally proposed by Olsson and Theander (Olsson and Theander 1976) and was thought to be particularly suited to the determination of the glucosinolate content of low glucosinolate rapeseed, rapeseed protein concentrates, and derived foods and feeds. The method has high sensitivity as only two peaks, α-D-glucose and ß-D-glucose are summed to obtain total gluco-sinolate content. Quantification is facilitated by the use of myo-inositol as internal standard. Relative to trimethylsilyl derivatives of de-sulfated glucosinolates, peaks for trimethylsilyl derivatives of glucose and myo-inositol have short retention times, but are well resolved. Background levels of glucose, from contamination of the myrosinase, may be avoided by dialyzing the myrosinase before use or corrected with appropriate controls.

4. ADVANTAGES/DISADVANTAGES

Gas chromatography of trimethylsilyl derivatives of glucose is a particularly sensitive method for determining glucosinolate content in low glucosinolate samples. Glucosinolate extraction and ion-exchange column purification steps lend themselves to batch type processing of samples and, along with short retention times of the chromatographic peaks, increase throughout (number of samples per day).

5. EQUIPMENT

Standard laboratory apparatus and in particular:

5.1 Culture tubes, 125 x 15 mm with PTFE-lined screw-cap:

Fisher Scientific Ltd.
Cat. No. 14-930-10E

5.2 Water bath, 100 °C (boiling).

5.3 Vortex mixer.

5.4 Centrifuge, bench top.

5.5 Disposable plastic syringes, 1 mL:

Fisher Scientific Ltd.
Cat. No. 14-823-226

5.6 Porous polyethylene sheeting, (Bel-Art) 1.6 mm 35 um pore size:

Mandel Scientific Co. Ltd.
Cat. No. F13638-5116

5.7 Cork bore, #1.

5.8 Oxford pipette tips, 1 mL:

Fisher Scientific Ltd.
Cat. No. 21-240-20

5.9 Disposable syringe needles, 20 guage, 38 mm long:

Fisher Scientific Ltd.
Cat. No. 14-826-5C

5.10 Holder for ion-exchange columns and corresponding holder for 4 mL vials.

5.11 Vials, teflon-lined screw-cap, 4 mL:

Canlab Ltd.
Cat. No. V3020-1 (vial)
Chromatographic Specialties Ltd.
Cat. No. C669112 (teflon liner)

5.12 Dry block heater, to accommodate 4 mL screw-cap vials, Reacti-Therm:

Chromatographic Specialties Ltd.
Cat. No. P18800

5.13 Compressed air and air maifold, Reacti-Vap Evaporator:

Chromatographic Specialties Ltd.
Cat. No. P18782

5.14 Hamilton syringes, 10 µL, 50 µL, 250 µL:

Chromatographic Specialties Ltd.
Cat. No. H80366 (10 µL) (fixed needle)
Cat. No. H80900 (50 µL) (fixed needle)
Cat. No. H81130 (250 µL) (Leur Lock removable needle)

5.15 Forced-air oven.

5.16 Gas chromatograph with flame ionozation detector:

Equipped with a 0.2 mm ID by 25 m fused silica capillary column coated cross-linked methyl-silicone 0.5 um thick.
Hewlett-Packard
Cat. No. 19091A 002
556-2-10A

6. REAGENTS

6.1 Acetic acid, glacial:

Fisher Scientific Ltd.
Cat. No. A38-500

6.2 Myrosinase:

Prepared from yellow mustard *Sinapsis alb*a L.) seed.
or
Biocatalysts Ltd.
or
Boehringer Mannheim Ltd.
Cat. No. 1088 769

6.3 N-Methyl-N-trimethyl-silylheptafluor (o) butyramide (MHSFBA):

Macherey-Nagel GmbH and Co.
Cat. No. 70126

6.4 Myo-inositol:

Sigma Chemical Co.
Cat. No. I-5125

6.5 Parafilm:

Fisher Scientific Ltd.
Cat. No. 13-374-5

6.6 Pyridine (silylation grade):

Chromatographic Specialties Ltd.
Cat. No. 27530

6.7 Sephadex DEAE A25:

Anion exchanger
40-120u bead size

Sigma Chemical Co.
Cat. No. A-25-120

6.8 Sodium hydroxide:

Fisher Scientific Ltd.
Cat. No. S318-100

6.9 Trimethylchlorosilane (TMCS):

Chromatographic Specialties Ltd.
Cat. No. 88530

7. SUPPLIERS

7.1 Biocatalysts Ltd.:

Main Avenue, Treforest Industrial Estate, Pontypridd, Wales CF37 5YT
Telephone: 044385 3712
Telex: 497126 BIOCAT G

7.2 Boehringer Mannheim Ltd.

11450 Cote de Liesse, Dorval, PQ CANADA H9P 1A9
Telephone: (514) 636-6760
Telex: 05-8222677

7.3 Canlab Ltd.

11620 181 St., Edmonton, AB CANADA T5S 1M6
Telephone: (403) 453-3921

7.4 Chromatographic Specialties Ltd.

P.O. Bag 1150, 300 Laurier Blvd. Brockville, ON, CANADA K6V 5W1
Telephone: (613) 342-4678

7.5 Fisher Scientific Ltd.

P.O. Box 3840 Station D
Edmonton, AB, CANADA T5L 4K2
Telephone: (403) 483-2123

7.6 Macherey-Nagel GmbH and Co.

Postfach 307, 5160 Duren
WEST GERMANY

7.7 Mandel Scientific Co. Ltd.

9840-47th Ave., Unit #2
Edmonton, AB, CANADA T6E 5P3
Telephone: (403) 436-0665

7.8 Sigma Chemical Co.

P.O. Box 14508, St. Lois, MO USA 63178
Telephone: (800) 325-8070
(314) 771-5750
Telefax: (800) 325-5052
(314) 771-5757

8. PREPARATION

8.1 DEAE Sephadex A-25:

- Weigh 10 g DEAE Sephadex A-25 into a beaker.
- Add 150 mL water and allow the Sephadex to swell overnight.
- Slurry onto a 20 x 400 mm column.
- Pass 500 mL 0.5 N sodium hydroxide (10 g dissolved in water and made up to 500 mL) through the column.
- Wash the column with 250 mL water to remove excess sodium hydroxide checking to ensure the pH has dropped to neutrality.
- Pass 400 mL 0.5 M pyridine-acetate (19.8 mL pyridine and 15 mL glacial acetic acid made up to 500 mL with water) through the column.
- Wash with 250 mL water.
- Slurry into a flask for storage.

8.2 DEAE Sephadex column, 0.2 mL:

- Cut a 1 mL plastic syringe at the 0.2 mL mark.
- Insert a disk of porous polyethylene cut from a sheet with a #1 cork bore into the shorter bottom piece of the syringe.
- Fill this part of the syringe ith DEAE Sephadex A-25, preswollen in water overnight.
- Cut a 1 mL Oxford pipette tip at the collar and insert the larger piece over the syringe tip filled with DEAE Sephadex.
- Place a 38 mm, 20 guage needle on the Luer lock tip of the syringe.

8.3 Myo-inositol, 1 mM:

- Weigh 45.1 mg into a 250 mL volumetric flask and make to volume with water.

9. PROCEDURE

9.1 Glucosinolate Extraction

- Weigh 100 mg of oil-extracted meal into a 125 x 15 mm culture tube.
- Place the tube in a boiling water bath for 1 minute.
- Add 2 mL of hot (<90 °C) water to the tube and, without allowing the contents to cool, mix to ensure that the meal is thoroughly wetted and continue heating for 3 min.
- Centrifuge at 2000 g for 10 min. and transfer the supernatant to a 5 mL volumetic flask.
- Wash the pellet twice with 1.5 mL of water, pool the supernatants, and make to volume with water.

9.2 Myrosinase Hydrolysis:

- Add 1 mL of the glucosinolate extract to a 0.2 mL DEAE Sephadex A25 column.
- Wash the column twice with 1 mL water discarding the washes.
- Transfer the column to a 4 mL vial covered with Parafilm to prevent evaporation.
- Add 0.1 mL of freshly prepared solution of myrosinase (3 mg/mL in water) collecting the column eleuate.
- Cap the column and let stand overnight at room temperature.
- Elute the column with 1 mL water pooling the eluate with the eluate collected upon addition of the myrosinase.

NOTE: It may be necessary to determine and correct for background glucose in the myrosinase preparation by setting up at least one additional column to which myrosinase is added, allowed to stand overnight and eluted as above.)

- Add 0.2 mL myo-inositol standard to the sample eluate and 0.05 mL myo-inositol standard and 0.15 mL water to the myrosinase background eluate.

9.3 Derivatization:

- Place the vial in a dry block heater at 60 °C and take to dryness by passing over the sample a stream of air, removing the vials when they are dry.
- Add in order, 100 µL pyridine, 100µL MSHFBA and 10 µL TMCS and immediately cap the vial.
- Heat at 120 °C for 20 minutes.

NOTE: It is essential that all reagents be dry. It is advisable to add the reagents to one sample at a time to minimize exposure to moisture in the atmosphere.

9.4 Chromatography:

- Inject approximately 1 µL of the derivatized sample onto the capillary column.

Carriergas(Helium)(mL/minute)..optimized
Air (mL/minute).....optimized
Hydrogen (mL/minute).....optimized
Detector range.....1
Detector attenuation.....16
Injector temperature(°C).....220
Detector temperature(°C).....300
Initial column temperature (°C).....180
Initial time (minute).....4
Program rate(°C/minute).....10
Final temperature(°C).....280
Final time (minute).....4

Approximate retenion times (minute)
α-D-glucose.....3.9
ß-D-glucose.....5.1
Myo-inositol.....6.5

10. CALCULATION AND REPORTING OF RESULTS

Area α-D-glucose + area ß-D-glucose/ area myo-inositol
*** 1.14285 * 0.2 * 5.0/1.0 * 1000/100 = µmoles/g oil-extracted meal**

where:

-**1.14285** is the ratio of the carbon number of the trimethylsilyl derivative of myo-inositol (24) to the carbon number of the trimethylsilyl derivative of glucose (21).
-**0.2** is the µmoles myo-inositol added to the sample.
-The factor **5.0/1.0** adjusts for the amount (1.0 mL) of the glucosinolate extract (5.0mL) placed on the ion-exchange column.
-The factor **1000/100** converts to a gram basis(1000 mg) the weight of oil-extracted meal (100 mg) from which the glucosinolates were extracted.

11. REFERENCES

MCGREGOR, D.I., W.J. MULLIN AND G.R. FENWICK. 1983. Review of analysis of glucosinolates. Analytical methodology for determining glucosinolate composition and content. J.Assoc. Off. Anal. Chem. 66: 825-849.

OLSSON, K., O. THEANDER AND P. AMAN. 1980. Determination of total glucosinolate content in rapeseed and turnip rapeseed meals by gas chromatography. Sewdish J. Agric. Res. 6:225-229.