COLLABORATIVE STUDIES ORGANIZED BY ISO AND THE COMMUNITY BUREAU OF REFERENCE (BCR-CEE) TO IMPROVE ANALYTICAL METHODS CONCERNING GLUCOSINOLATES IN RAPESSEED

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INTRODUCTION

Glucosinolates (GLS) are limiting the use of rapeseed cake. Some GLS reduce the palatability of the cake (leading to reduce animal intake), others are known to interfere with thyroid function, to damage vital organs or interfere with metabolic processes. There is thus a move towards rapeseed which is low in glucosinolates.

Close collaboration is now well established between the International Organization for Standardization (ISO) and the Community Bureau of Reference (BCR-CEE) to improve and compare different glucosinolate methods (organization of international ring tests, preparation of certified reference materials...).

The first part of this paper shows the results of a ring test organized in summer 1990 by ISO. Three rapeseed samples has been analysed by HPLC of desulphoglucosinolates using sinigrin and glucotropaeolin as internal standard. The total glucosinolate content has also been determined by X-ray fluorescence (XRF).

The second part gives informations concerning the preparation by the BCR of three reference materials with a certified content in total glucosinolate content and in sulphur (for XRF calibration).

MATERIALS AND METHODS

a) Ring test ISO

Seeds are in heat sealed laminated bags (20 g). Sample A is a German variety, sample B a French variety (Darmor) and sample C is a Canadian variety.

The HPLC method is the ISO DP 9167 which is very similar to the official EEC method (Journal of the European Communities,n° L170/28 03/07/90). Glucosinolates are extracted with hot methanol (70/30), desulphated with Helix pomatia, cleaned on a DEAE sephadex A 25 resin and injected in the HPLC.

The X-RF method measure the total sulphur content. The GLS level is defined as the total sulphur content minus those amounts that are bound in proteins or single GLS that cannot be determined by direct reference methods, divided by the average stoichometric number of sulphur atoms occuring in the GLS fraction typical of brassica species. Calibration has been made with three reference materials with a know content in sulphur prepared by the Commission of the European Communities (BCR programme), Brussels.The theoretical principles of the XRF method has been published by Schnug (Schnug E. and Haneklaus 1988; Schnug et al. 1990).

Determination of glucosinolate content must be carried out

on two separate determinations employing each time the proposed methods.

b) Reference materials

RM 366 is a german variety, RM 190 and RM 367 french varieties (Darmor and Jet Neuf). Light impurities (leafy materials, stalks, etc.) were removed by blowing air through the seeds. Large foreign matter was removed manually. 50 Kgs of each material were separately mixed in a single batch in horizontal blender fitted with helical blades. The materials were blended for 45 minutes; prolonged was avoided since it could lead to fracture of the whole seed. After confirmation that the batches were homogenous, the seeds were packed under nitrogen in units of 20g in heat sealed aluminium plastic laminated sachets.

Methods for the certification of total glucosinolate content are the HPLC of desulphoglucosinolates (EEC Journal n° L170/28 03/07/90), HPLC of intact glucosinolates (Helboe et al. 1980), gas liquid chromatography (Heaney et al. 1986), glucose release method with hexokinase (Heaney et al. 1986) and a sulphate method (Schnug 1988).

The sulphur has been measured by IDMS, ICP and ion-chromatography with a variety of sample digestion procedures.

RESULTS

INTERCOMPARISON OF METHODS

The table 1 shows that the use of sinigrin as internal standard in the HPLC method increase slowly the total glucosinolate content. This may be due to response factors, integration or desulphatation problems.

Repeatability is better with sinigrin but the reproducibility remains similar.

The mean observed by X-RF is lower than by HPLC (+/- 2 µmol/g).The equation proposed in the method to calculate the GLS content must be slightly changed. Repeatability and reproducibility with the XRF are very nice, better than by HPLC. We believe that it is the results of the use of similar reference materials to calibrate the equipment.

BCR REFERENCE MATERIALS

The certified reference materials allow any laboratory to verify its measurements but also in some cases to calibrate a procedure (e;g; calibration of XRF equipment with rapeseed CRMs of known S content).

a) Homogeneity of the packaged material

For each variety, sachets were taken at regular intervals of the filling sequence (systematic sampling).

* The total GLS content of the seeds of each sachet has been determined in one laboratory by HPLC of the desulphoglucosinolates on 0.5g samples.

Table 1. HPLC results for laboraties who have used two different internal standards (sinigrin SIN and glucotropaeolin TROP)(total glucosinolate content)

	sample A		sample B		sample C	
	SIN	TROP	SIN	TROP	SIN	TROP
Number of laboratories selected after elimination of the abnormalities	7	7	7	7	7	7
Mean (µmol/g)	18.6	17.9	34.7	33.9	13.3	13.1
Repeatability standard deviation	0.8	1.2	1.3	2.2	0.6	1.0
Repeatability variation coefficient (%)	4.3	6.4	3.7	6.5	4.1	7.6
Repeatability	2.2	3.3	3.6	6.2	1.6	2.8
Reproducibility standard deviation	2.2	2.7	5.0	5.2	1.8	1.4
Reproducibility variation coefficient	11.8	15.2	14.4	15.3	13.6	10.7
Reproducibility	6.2	7.7	14.1	14.6	5.1	4.0

Table 2. X-RF results (total glucosinolate content)

	sample A	sample B	sample C
Number of laboratories selected after elimination of the abnormalities	20	20	20
Mean (µmol/g)	15.5	31.3	11.4
Repeatability standard deviation	0.4	0.9	0.3
Repeatability variation coefficient (%)	2.7	2.8	2.7
Repeatability	1.2	2.5	0.9
Reproducibility standard deviation	0.7	1.5	0.5
Reproducibility variation coefficient	4.3	4.7	4.3
Reproducibility	1.9	4.2	1.4

Table 3. Homogeneity of the packaged material (glucosinolate content)

	RM 366	RM 190	RM 367
Number of sachets	20	23	20
MEAN (μmol/g)	13.71	25.20	104.05
Standard deviation	0.84	0.90	1.63

No evidence of a gradient corresponding to the filling sequence was detected in the materials. The coefficients of variation of the results for the total glucosinolate contents was 3.5% for RM 366, 6.7% for RM 190 and 1.6% for RM 367 which correspond to the repeatability of the method used at these contents. It was concluded that the batches were acceptably homogeneous for sample intakes of at least 0.5 g.

* The homogeneity of the RMs was also examined by wavelength dispersive XRF measurement of the sulphur content of the packaged samples.The repeatability of the XRF method was evaluated by replicate analysis of a thoroughly mixed batch of 0.2 Kg rapeseed. The sample was divided into 10 parts from each of which 3 discs were prepared and the sulphur content determined in each of them. The coefficient of variation was found to be 0.6 % at a sulphur content of 4.3 mg/g.

Table 4. Homogeneity of the packaged material (S content)

		RM 366	RM 190	RM 367
Number of sachets		12	12	12
MEAN mg/g	between discs	3.178	4.574	9.552
	between sachets	3.178	4.574	9.552
	within sachets	3.178	4.574	9.552
Standard deviation				
	between discs	0.033	0.053	0.107
	between sachets	0.022	0.041	0.031
	within sachets	0.025	0.034	0.103

Application of the variance ratio test to the within and between sachets variances gave no indication of significant differences. No evidence of significant inhomogeneily of the sulphur content was detected in any of the batches.

b) Stability of the GLS content of the seeds

Stability of RM 190 stored at -4°c under nitrogen has been studed by one laboratory which made single measurements by HPLC at each point on 200 mg samples. No evidence of change (Table 5) of total glucosinolate was found after 30 months. RM 366 and RM 367 were also subjected to a restricted study to confirm the expectation of stability (Table 6). No change appears after 60

weeks. The stability ot the reference material will be subjected to continuous monitoring.

Table 5. Influence of storage time on total GLS content (μmol/g) of RM 190 at 4-6°c under nitrogen

	time of storage in months						
T°	0	3	6	12	18	24	30
4-6°c	24.65	24.45	24.85	24.80	24.80	24.71	24.66

Table 6. Influence of storage time on total GLS content (μmol/g) of RM 366 and RM 367 at 4-6°c under nitrogen.

RM	Method Repeatability	Duration of study (weeks) before packaging	after packaging		
		0	12	40	60
RM 366	+/- 0.84	12.9	12.2	12.8	13.2
RM 367	+/- 1.63	101.0	99.5	100.2	100.5

c) Certification exercise for Total GLS content.

The certified value is based on measurements by experienced European laboratories and a variety of methods (HPLC of desulphoglucosinolates, HPLC of intact GLS, gas liquid chromatography, glucose release method and sulfate method).Each participant was required to make a total of 5 separate determinations on samples taken from 2 sachets of each reference material.

The results accepted for certification of the total GLS content are presented in table 7. Indicative values for individual GLS has also been determined. CRM are available from the BCR in units of 20 g.

Table 7. Total glucosinolate content (μmol/g) Certified values

RM	Mean	uncertainty (95% confidence level)	n° of accepted sets of results
RM 366	12.1	+/- 0.8	18
RM 190	25.5	+/- 0.9	16
RM 367	102.0	+/- 3.0	16

d) Certification exercise for total sulphur content (mg/g)

The methods used for this exercise are IDMS, ICP and ion-chromatography with a variety of sample digestion procedures. Calibration was based on compounds of known purity or stoichiometry and, in several cases, the methods were validated by analysis of materials of known sulphur content before certification measurements were made.

The certified sulphur contents are presented table 8.

Table 8. Total sulphur content (mg/g)
Certified values

RM	Mean	uncertainty (95% confidence level)	n° of accepted sets of results
RM 366	3.41	+/- 0.12	4
RM 190	4.93	+/- 0.15	4
RM 367	10.50	+/- 0.40	4

CONCLUSIONS

Close collaboration is now well established between ISO and the Community Bureau of Reference . A ring test shows that repeatability is better by XFR (with use of reference materials) than by HPLC .Repeatability is better with sinigrin than with glucotropaeolin as internal standard (HPLC method)

Reference materials with a known content in total GLS or sulphur has been prepared with succes.

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