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Rapeseed reference materials for glucosinolate analysis

Development of rapeseed BCR RM 190 and the results of the intercomparison of methods

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Rapssamen-Referenzmaterial für die Glucosinolatanalyse. Entwicklung von Rapeseed BCR RM 190 und Ergebnisse eines Ringversuchs

Summary. The use of rapeseed cake is limited by the presence of a number of antinutritional factors the most important of which are glucosinolates. Introduction by EEC of a premium to produce rapeseed with low glucosinolate content has brought the need for reference material and accurate official methods of analysis. This paper summarises the work, begun at the end of 1987, to prepare three batches rapeseed materials (cleaning, mixing and packaging) for an intercomparison of methods. Details are presented of homogeneity and stability studies and the results of the intercomparison are discussed. One of the batches was prepared in sufficient quantity to serve as a reference material and the reference value for the total glucosinolate content of this sample was obtained from the results of the intercomparison.

Introduction

Rapeseed is virtually the only oil seed which can be grown economically in the northern latitudes. It is cultivated mainly as a rich source of valuable edible oil, the seed having an oil content of about 40%.

The cake which remains after the oil is expelled from the seed is widely used as a feed for farm animals and poultry. Although a rich source of protein (36%–40%) and fibre (12%–14%), the use of rapeseed cake is limited by the presence of a number of antinutritional factors the most important of which are glucosinolates (GSL). Certain GSL reduce the palatability of the cake (leading to reduced 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 GSL. The Community actively encourages increased production and usage of rapeseed cake as a means of reducing animal feed imports and of moving production away from crops grown in surplus. The Community currently pays a premium to producers of rapeseed which is low in GSL. At the present time, the limit is 35 μmol/g but this will fall to 20 μmol/g.

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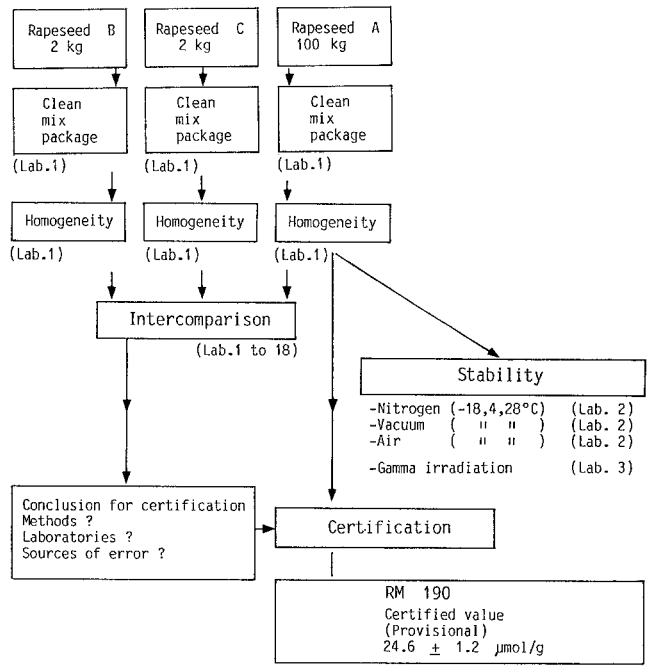
Introduction by the EEC of a premium payment to producers of low GSL rapeseed has brought the need for an accurate, official method of analysis and for reference materials (RMs). It is in the context of the need for a RM that the Community Bureau of Reference (BCR) has undertaken a collaboration project, the results of which are now presented.

In the first part of the paper, the preparation of materials is described and in the second part, the results of the recent intercomparison of methods are presented and discussed.

Experimental procedure

Three different rapeseed materials (A: ± 25 μmol/g, B: ± 75 μmol/g and C: ± 12 μmol/g) were cleaned, mixed, packaged in aluminium/plastic laminate sachets (20 g), labelled and stored at 4° C. Homogeneity was confirmed by high performance liquid chromatography (HPLC) and gas liquid chromatography (GLC) of desulphoglucosinolates, and by glucose release (GLUC), the palladium test (PALL) and near infrared spectroscopy (NIR).

Table 1. Scheme of work



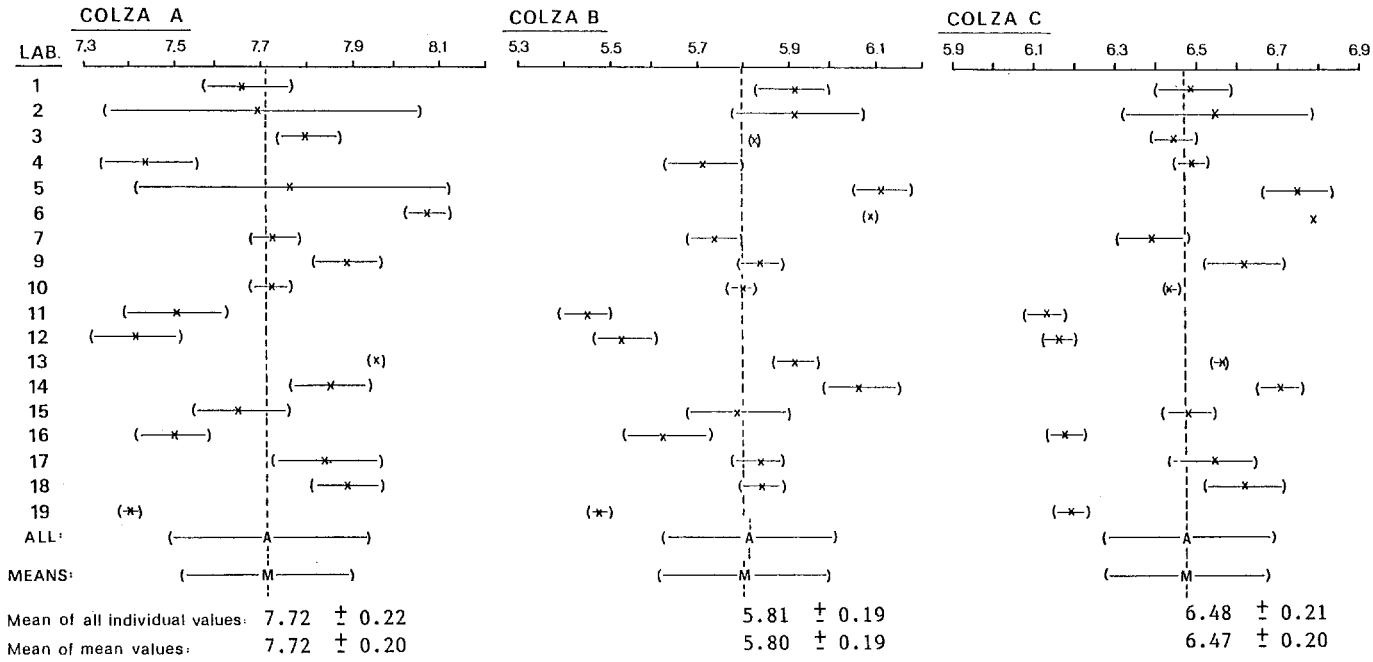


Fig. 1. Water content of rapeseed by oven drying at 103° C (g/100 g). Bar graphs for laboratory means and standard deviations

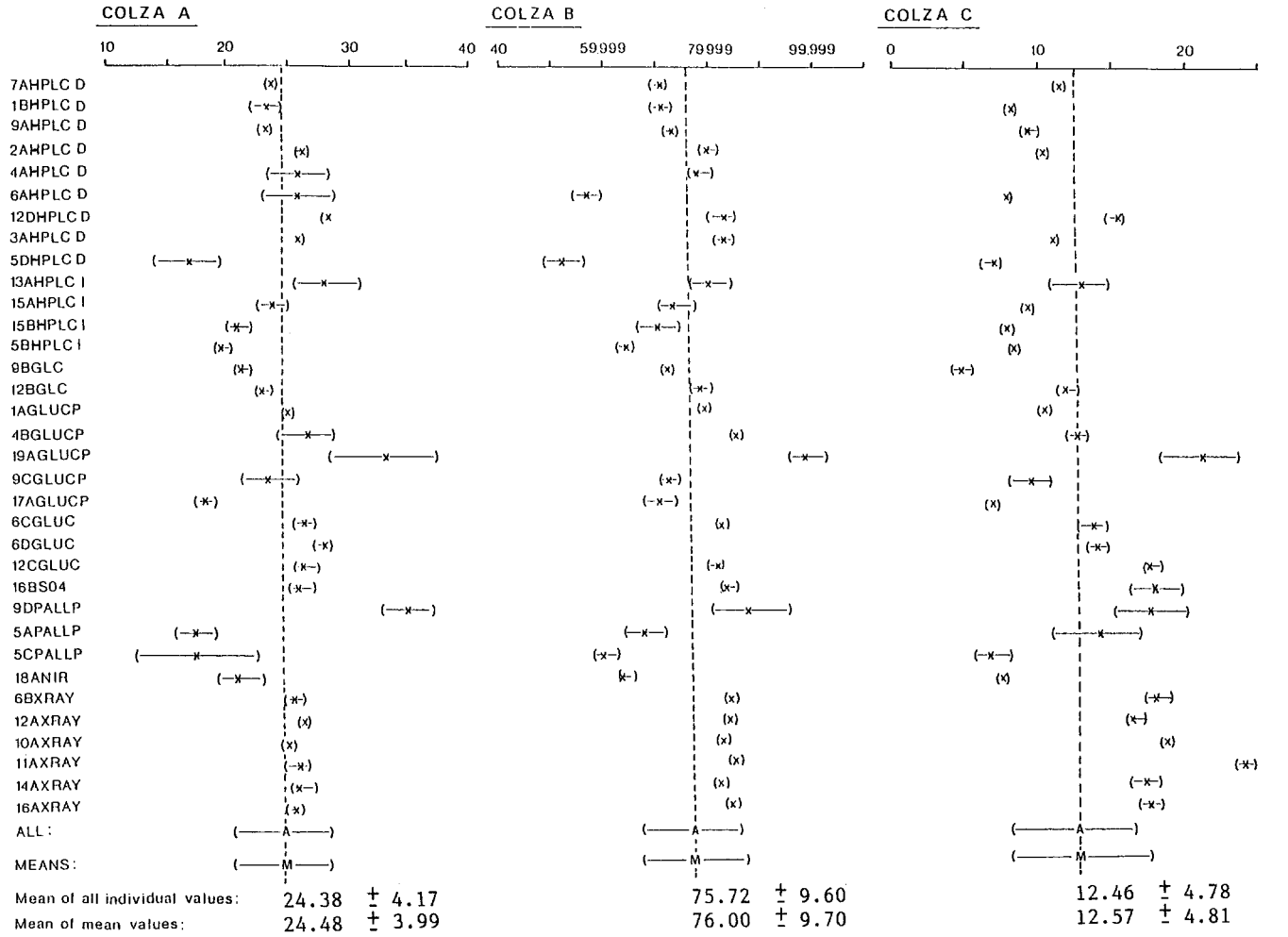


Fig. 2. Total glucosinolate content. Bar graphs for laboratory means and standard deviations

Table 2. Summary of methods

Code	Extraction solvent	Grinder	Purification column	Separation column	Internal standard	Wavelength oven temp.
HPLC desulphoglucosinolates						
7AHPLCD	H_2O	cof. mill	DEAE A 25	Lichrosorb 5RP18	tropaeol.	229 nm/room t°
1BHPLCD	MeOH/H_2O 70%	cof. mill	DEAE A 25	Spherisorb ODS2	tropaeol.	229 nm/30° C
9AHOLCD	MeOH/H_2O 70%	u. turrax	DEAE A 25	Spherisorb ODS2	tropaeol.	229 nm/30° C
2AHPLCD	MeOH/H_2O 70%	cof. mill	DEAE A 25	Lichrospher 100RP8	sinigrin	229 nm/35° C
4AHPLCD	MeOH/H_2O 50%	cof. mill		Lichrosorb Merck	sinigrin	229 nm/20° C
6AHPLCD	MeOH/H_2O 70%	cof. mill	DEAE A 25	Nucleosil	sinigrin	229 nm/30° C
12DHPLCD	MeOH/H_2O 70%		DEAE A 25	RP 18 5μ	sinigrin	229 nm/30° C
3AHPLCD	MeOH/H_2O 70%	cof. mill	DEAE A 25	Novapack C 18	tropaeol.	229 nm/26° C
5DHPLCD	MeOH/H_2O 70%	u. turrax	DEAE A 25			230 nm
HPLC intact glucosinolates						
13AHPLCI	MeOH/H_2O 70%	u. turrax	Ecteola	Spherisorb S3ODS2	sinigrin	235 nm
15AHPLCI	EtOH/H_2O 70%	u. turrax	–	Spherisorb S5ODS2	sinigrin	235 nm/40° C
15BHPLCI	EtOH/H_2O 70%	u. turrax	Ecteola	Spherisorb S5ODS2	sinigrin	235 nm/40° C
5BHPLCI	MeOH/H_2O 70%	u. turrax	QMA	Spherisorb S3ODS2		235 nm
Gas-liquid chromatography						
9BGLC	MeOH/H_2O 70%	u. turrax	DEAE A25	OV 7 2% diatomite	sinigrin	
12BGLC				OV 101	sinigrin	
Glucose with purification on an ion-exchange column						
1AGLUCP	MeOH/H_2O 70%	cof. mill	DEAE A 25			520 nm
4BGLUCP	MeOH/H_2O 50%	cof. mill	Bond Elut			530 nm
9CGLUCP	MeOH/H_2O 70%	u. turrax	DEAE A 25			520 nm
Glucose without purification on an ion-exchange column						
6CGLUC	H_2O	cof. mill				340 nm
6DGLUC	H_2O	cof. mill				
12CGLC	MeOH/H_2O 70%					240 nm/25° C
Sulfate method						
16BSO4	H_2O	cof. mill				
Palladium method						
9DPALLP	MeOH/H_2O 70%	u. turrax	Ecteola		sinigrin	425 nm
5APALLP	MeOH/H_2O 70%	u. turrax	Ecteola		sinigrin	425 nm
5CPALLP	MeOH/H_2O 70%	u. turrax	DEAE A 25		sinigrin	425 nm
Near infrared						
18ANIR					1st der.	1624, 1636, 1640
X-ray fluorescence						
6BXRAY						$S\text{-}K_a$
12AXRAY						
10AXRAY						$S\text{-}K_a$
11AXRAY						
14AXRAY						75.83
16AXRAY						5.372 A°

The essential steps are summarised schematically in Table 1.

Rapeseeds A, B, C were used for a first intercomparison of methods involving eighteen laboratories. The choice of the method was left to the participants with the requirement that they applied their method(s) with the highest degree of accuracy and precision, paying particular attention to calibration.

The stability of glucosinolates contained in rapeseed A is being studied by a second laboratory under different packaging conditions: nitrogen, vacuum or air at three temperatures (−18; 4 and 28° C). A third laboratory is investigating the stability of GSLs after gamma irradiation.

At the time of writing, there is no evidence of instability under the selected storage conditions.

Results of the intercomparison of methods

A Moisture content

Each laboratory made four determinations of the moisture content of each sample according to a standardized method (oven drying of 2 ± 0.2 g of the sample for 3 h at 103 ± 2° C under atmosphere pressure and then for 1 h periods to constant weight). The results are shown in Fig. 1, where "×" is the mean observed and "( )" represents the SD.

Table 3. HPLC response factors

	Desulphoglucosinolates		Intact glucosinolates		
	EEC method	5DHPLCD	13AHPLCI	15AHPLCI 15BHPLCI	5BHPLCI
	229 nm	230 nm	235 nm	235 nm	235 nm
Sinigrin	1.00	1.00	1.00	1.00	1.00
Gluconapin	1.11	1.10	1.09	1.09	1.10
Glucobrassicanapin	1.15	1.10	1.07		1.10
Progoitrin	1.09	1.10	1.04	1.16	1.10
Epiprogoitrin	1.09	1.10			1.10
Napoleiferin	1.00	1.10	1.06	1.06	1.10
Glucoiberin	1.07	1.20			1.20
Glucoraphanin	1.07	1.20			1.20
Glucoalyssin	1.07	1.20			1.20
Glucotropaeolin	0.95	0.95		0.88	0.95
Gluconasturtiin	0.95	0.95	1.00		0.95
Glucobarbarin	1.00	0.95			0.95
Sinalbin	1.00	0.55			0.55
Glucobrassicin	0.29	0.45	0.45	0.45	0.45
Neoglucobrassicin	0.20	0.35	1.00	0.37	0.35
4-OH glucobrassicin	0.28	0.30	0.27	0.27	0.30
4-Methoxyglucobrassicin	1.00	0.30			0.30

Laboratories 6, 13 and 14 found systematically higher values and laboratories 11, 12, 16 and 19 lower values for the three materials. It was concluded that the discrepancies would not significantly influence the total glucosinolate value when the latter was expressed on a dry mass basis.

B Total glucosinolate content

Essential details of the methods used by the laboratories are summarised in Table 2, with corresponding response factors employed for HPLC being given in Table 3.

The results, grouped by method, are presented in bar-chart form (mean $\pm$ 1 SD) in Fig. 2.

In Table 2 and Fig. 2, the results are given with the laboratory code (7A, 1B, etc.) with the method type denoted by the following alpha-numeric codes (references to the methods are given in brackets).

HPLCD: HPLC desulphoglucosinolates [3, 10]; HPLCI: HPLC intact glucosinolates [2, 5]; GLC: gas liquid chromatography of desulphoglucosinolates [4]; GLUCP: glucose release with purification [4]; GLUC: glucose release without purification [11]; SO_4: sulphate method [7, 9]; PALLP: palladium colourimetric method with purification [6]; XRAY: X-ray fluorescence [8]; NIR: near infrared spectroscopy [1].

Direct methods

With the exception of the results of laboratory 5 which were systematically low for all methods and samples, HPLC of desulphoglucosinolates and of the intact compounds were close to the general mean.

The results of the two GLC methods (labs 9B and 12B) showed that accurate results could be obtained if steps were taken to avoid break-down of 4-OH glucobrassicin and certain S containing GSLs because of high injection temperatures. This source of error becomes increasingly important for low GSL rapeseed such as sample C where the proportion of 4-OH glucobrassicin is relatively large (30%) c.f. sample A where it is about 12%.

With the exception of the results of lab. 19, the glucose release method with purification on an ion-exchange column to remove free glucose gave results which were in good agreement with those of the HPLC methods. In the absence of a preliminary clean-up, it is essential to make a blank correction for free glucose.

This effect is illustrated by the results 6C GLUC and 12C GLUC where a correction was made, and by the higher results of 6D GLUC where it was not.

A novel sulphate method was also used by laboratory 16. This method [7, 9] considered to be virtually free from interference by non-GSL compounds gave results in line with the overall mean.

As observed in previous studies, results by palladium methods gave high with- and between-laboratory variance. This method is widely accepted as being insufficiently specific and quantitation is difficult because individual GSLs give different colourimetric responses.

Indirect rapid methods

Several laboratories used rapid, indirect methods based on NIR and XRF.

NIR is a very rapid (1 sample per minute) and inexpensive method but was only applied by one laboratory (19). The equipment was calibrated on the basis of the HPLC method (desulphoglucosinolates) carried out by lab. 9. The correlation coefficient for the calibration curve was 0.99 and the standard error of the estimated values was 2.15 μmol/g. The NIR results were systematically lower than the overall mean suggesting that a modification of the calibration curve is necessary. NIR offers the advantage of simultaneous determination of protein, water, lipids and fibre content.

The six sets of results by XRF (S determination) are characterised by remarkably good within- and between-laboratory variance. Reproducibility was achieved by the use of a common set of rapeseed calibrants by each of the six laboratories and demonstrates the effectiveness of the use of reference materials in achieving a high level of comparability.

The XRF values are generally consistent with the overall mean for samples A and B but were rather high for the low GSL rapeseed C. The usefulness of the XRF method depends on the fact that rapeseed has an almost constant level of non-glucosinolate S containing compounds (mainly S amino acids). It is to be expected that XRF will tend to give higher results than HPLC because it additionally determines S containing GSL breakdown products.

Rapeseed reference material (BCR RM 190)

Rapeseed A had been prepared in sufficient amounts to serve as a reference material for GSL analysis. Technical evaluation of the results for total glucosinolate obtained by direct methods (HPLC, GLC and glucose release and sulphate methods) has led to a provisional certified total glucosinolate content of (24.6 ± 1.2) μmol/g. Samples of the reference material will shortly be available from the BCR in units of 20 g.

Conclusions

Taken overall, HPLC, GLC glucose release and SO_4 methods show a very good level of agreement with no obvious sign of bias in any of these procedures.

The palladium procedure gave poor results and is not considered suitable for this assay.

XRF methods had very good reproducibility but tended to be higher than the overall mean, especially for the low glucosinolate sample C. The single set of NIR results had good repeatability but was a little lower than the overall mean, suggesting that the calibration required improvement.

The study has led to the availability of a rapeseed reference material with a total glucosinolate content of (24.6 ± 1.2) μmol/g.

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