The Analysis of Glucosinolates in Rapeseed

The Current Situation

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This paper does not attempt to review critically the many methods which have been developed for glucosinolate analysis. Instead, a number of areas where glucosinolate analysis is important are considered and an overview of the current rather complex situation is presented.

The question of glucosinolate analysis is viewed from different perspectives by, for instance, the rapeseed breeder, European legislator or feed compounder. A recent review (1) of the literature on glucosinolate analysis shows that few if any analytical developments over the last 100 or more years have not been applied to glucosinolates and their products. Of course many methods are outdated, being replaced by techniques which are increasingly automated and (micro) processor controlled. However, 'new' methods continue to be published with no fewer than 27 papers describing such new (or modified) methods in the last two years alone. It is against this background that the analysis of rapeseed will be discussed under 3 headlings :

a) methods suitable for the breeder,
b) methods linked to present/forthcoming legislation and, since the foregoing refer to rapeseed,
c) methods for the analysis of rapeseed meal.

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a) Methods suitable for the breeder

For nutritional reasons the removal (reduction) of glucosinolates in rapeseed has been, and remains, the primary goal of plant breeders and spectacular advances have been made. Such programmes must be monitored by means which provide the necessary discrimination, simplicity, reliability and inexpensiveness and which determine all glucosinolates. Methods based upon measurement of the glucose released after treatment with myrosinase are frequently used and cheapness has been made a virtue in the Tes-stick and Tes-tape approach of Canadian workers. There was much discussion at the Rapeseed Congress in Paris in 1983 of the Palladium method introduced by Professor Thies (2) and developed amd automated by Dr. MacGregor. This method which has still not been compared in detail with other (validated) methods (glucose release; GC), has been modified and adopted by Professor Sørensen in Copenhagen (details available from the author and in the Proceedings of an EC Conference on Rapeseed, Copenhagen 1984, (3)).

b) Observations on Glucosinolate Analysis methods linked to present/forthcoming legislation.

The Canadian Grain Commission has developed a format for certification of glucosinolate levels in rapeseed cargoes. Certification mark (No. 243,139), registered 18.4.80 includes the following definition of Canola, w.r.t. glucosinolate content "shall be the seed of the species *Brassica napus* or *B. campestris*... the solid component of which contains less than 30 μm of any one or any mixture of 3-butenyl glucosinolate, 4-pentenyl glucosinolate, 2-hydroxy-3-butenyl glucosinolate and 2-hydroxy-4-pentenyl glucosinolate per gram of oil-free, air-dry solid". The points to note here are the exclusion of indole glucosinolates and the units, per gram defatted meal. Clearly such a definition must be underpinned by specification of an analytical method if it is to have any validity and meaning. The method specified (Canadian Grain Commission, Daun and McGregor 1981, amended 1983 (4)) is that of temperature-programmed GC, following extraction of the glucosinolates from defatted meal, enzymatic desulphation and volatilization. The method is based on that of Heaney and Fenwick (5), which in turn was an extension of the method of Thies (6). Thies' original procedure utilized isothermal GC, and consequently indole glucosinolates were not observed. Operation under temperature-programmed conditions allows indole glucosinolates (glucobrassicin and 4-hydroxy-

glucobrassicin) to be separated and quantified. Thus the Canadian Grain Commission method separates all glucosinolates but then leaves the indoles out of the calculation. The rationale behind this omission is that (i) there is no evidence that these cause nutritional or toxic problems in animals and (ii) in licensed Canadian Canola cultivars, levels are constant at 10-14 μm/g. There appears to be no published data demonstrating the lack of toxic or antinutritional effect of indole glucosinolates and until this is presented and accepted 'glucosinolate content' should mean just that. Secondly, indole glucosinolate content may have values much wider (down to 2 μm/g, up to 30 μm/g also being proportionally greater in 00 than 0 seed) than those reported from Canadian material.

In general support of the argument that indole glucisonolates should be included, the EC Committee of Experts on the Analysis of Glucosinolates in Rapeseed have recently put forwards an Interim method, which is based upon the Canadian method but includes the contribution of indoles (7). Moreover the ISO, via AFNOR having recently ring-tested an isothermal method (ie. excluding indoles) are now embarking upon a second exercise which will examine a temperature-programmed method with a view to introducing this as an official ISO method. Unfortunately although similar in principle the Canadian, EEC interim and proposed ISO methods all differ in detail as shown in Table 1. Glucosinolate contents are calculated differently and at least as originally conceived in the EEC version, expressed differently.

Many workers now extract glucosinolates directly from the ground full fat seed, without an initial defatting procedure. The latter, of course, can prolong the assay and introduce error. This being the case it has been persuasively argued that expression of glucosinolate content should be as μm/g seed (defined moisture content) rather than μm/g meal. Due to the need to introduce the EC interim method in 1985 the Experts meeting decided that it would be wrong to depart in this respect from the defatting procedure laid down in the Canadian Grain Commission method hence expression of content in μm/g meal is presently acceptable, but it is considered that, with the introduction of direct extraction from seed, this expression may be changed during (and certainly will be after) the interim period. There seems to be general agreement, that HPLC methods will eventually replace todays GC methods, the elimination of the derivatization step being but one advantage. When this will come is less easy to guess. The EC timescale for this change is 2-3 years, but 4-5 years is likely to be nearer the correct figure, which would be appropriate, 1990 – ushering in the new decade of glucosinolate research. Whether the HPLC method will be based on the measurement of intact or desulphoglucosinolates (both having their proponents (8) (9)) is yet to be decided. The EC is currently evaluating and comparing these methods on standardized rapeseeds. There is also the problem of response factors and implicit in this is the question of the availability of standards. There are also questions about the stability of the main indole glucosinolate, 4-hydroxyglucobrassicin, under the conditions of the extraction/

Table 1 – Main differences between different g.c. analytical methods

	Canadian GC	Interim E.C.	I.S.O.
Internal standard	Glucotropaeolin or Sinigrin	Sinigrin	Glucotropaeolin or Sinigrin
Derivatization Reagent	MSHFBA Pyridine TMCS	MSHFBA 1-Methylimidazole TMCS	MSHFBA Pyridine TMCS
Buffer	Pyridine acetate	Pyridine acetate	Imidazole formate
Defatting step			
Column Conditions	180° for 5 min 5°/min 280°	200° for 5 min 5°/min 280°	200° for 5 min 5°/min 280°/min
Response Factor	Carbon Number	Unity	Unity

analysis. Conditions for optimal recovery of this compound may need to be defined (10). It is to be hoped that uniformity of glucosinolate analysis may come about in this area, with the advent of HPLC and that contacts between national and international bodies and large organizations (as well as between the analysts themselves) will facilitate this.

c) Methods for the analysis of rapeseed meal

Many methods have been reported and patented for the detoxification of rapeseed (and its products). Detoxification means primarily the removal of glucosinolates and hydrolysis products. The prevailing view is that double zero rapeseed will make relatively little impact in the UK before the end of the decade (at least) and additional processing is being actively considered. Extrusion, micronization, heat and solvent processing and addition of solid and gaseous chemicals are all being explored.

In some, though not all of these processes, the implicit assumption is made that removal of glucosinolates per se equates with detoxification. That this is not necessarily so is shown in Table 2. Processes D,E look particularly promising compared with the original seed meal A. However if breakdown products are included the picture is very different – with high levels of hydroxynitriles being produced – especially in C, D and E. Thus the monitoring of the contents of glucosinolates and their breakdown products is necessary. Moreover products need not necessarily parallel those expected from myrosinase treatment (oxazozalidine-2-thiones; nitriles and isothiocyanates) – it is known for example that the presence of ferrous salt can produce thioamides rather than the above, more common products. These areas are clearly ones where the analyst, the nutritionist, the processor and the breeder can work closely together to ensure the quality of the product. It is the quality of the product which after all will dictate the market success of rapeseed and its products.

Although the methods described here do not represent the ultimate in precision and sophistication, their sensitivity and accuracy can only be put into context when the physiological, antinutritional and clinical properties of glucosinolates and their products are fully understood.

Table 2 – The effect of processing on the content of progoitrin and its breakdown products in rapeseed meal

Process	Glucosinolate* content	Progoitrin* content	OZT** content	Hydroxynitrile** content
A	100 (57 m)	100 (24 m)	–	–
B	82	73	1.3	2.9
C	57	46	1.9	7.7
D	15	12	1.9	16.4
E	11	5	2.3	19.2

* % original
** m/g defatted meal

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