A Revision to the Canola Definition

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The concept which resulted in the development of canola began in the 1960's with the realization that a major drawback to complete utilization of rapeseed meals in animal rations was the presence of significant quantities of glucosinolates. Plant breeders had successfully modified of the fatty acid composition of rapeseed, with Dr. Downey releasing the first low erucic acid variety *B. napus* cv. Oro, in 1968. A year earlier, in 1967, Professor Jan Krzmanski, working at the Agriculture Canada Research Station in Saskatoon isolated the low glucosinolate rapeseed variety *B. napus* cv. Bronowski. This work was accomplished using a screening method for glucosinolate analysis developed at the NRC Laboratory in Saskatoon. [1]

Continued development of low glucosinolate rapeseed varieties took a lower priority in the early 1970's after research presented at the International Rapeseed Conference in St. Adèle, Quebec suggested that erucic acid may be undesirable when consumed in large quantities. In 1973, the Canadian rapeseed industry established voluntary guidelines for the level of erucic acid (5%) contained in rapeseed oil products produced in Canada. At the same time the industry considered introducing the name Canbra to designate this new product but this name was not readily accepted by most of the industry after it was chosen as the name of a new processing company.

Breeding efforts to develop low glucosinolate varieties continued and in 1975 *B. napus* cv. Tower, the first variety with low levels of both erucic acid and glucosinolates was released by Dr. Stefansson at the University of Manitoba. This was followed, in 1978 years later, by the release of Candle, the first *B. rapa* variety with low levels of glucosinolates and erucic acid.

The presence of varieties from both species with low levels of glucosinolates and erucic acid meant that the Canadian crop would soon be converted to this new type of seed. Recognizing the vast improvement in quality that these new varieties offered over the old rapessed types, the Canadian industry decided to rename the new commodity as "canola". [2]

On September 8, 1978, the Rapeseed Association of Canada filed an application for registration of a certification mark for the word "canola". The mark was certified on April 18, 1980. The registration stated, in part that canola [3]

> "..shall be the seed of the species Brassica napus or Brassica campestris, the oil content of which seed containts less than 3 milligrams of glucosinolate per gram of solid (GLC Method-MacGregor)"

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The measurement system used for glucosinolates used in this original definition was based on the analytical method developed for plant breeding. This method, for simplicity, reported the glucosinolate content of rapeseed in terms of milligrams per gram of the hydrolysis products produced when the glucosinolates were hydrolyzed in the presence of the enzyme mysrosinase. As butylisothiocyanate was used as the reference compound in the GLC measurement of the isothiocyanates formed in the hydrolysis step, it became customary to express results as "mg/g or butylisothiocyanate". Unfortunately the hydrolysis product, in this case, makes up only about 1/3 of the actual glucosinolate weight and thus the wording of the definition restricted the maximum level of glucosinolates in canola to only 1/3 of what was intended[1].

The use of "mg/g" or % or other weight/weight units to express glucosinolates, even as intact glucosinolates may also cause difficulties because of the different molecular weights of the glucosinolates present in canola seed. This means that the same weight of different glucosinolates will have different numbers of biologically active units (molecules) (Table 1).

Table 1. Effect of expressing glucosinolates on a weight basis on the number of molecules (moles).

Glucosinolate Ion "R" Group	Molecular Weight	Hydrolysis Product	Molecular Weight	Micromoles in 3 mg	mg in 30 Micromoles
allyl-	342	isothiocyanate	99	30.3	3.0
3-butenyl-	355	isothiocyanate	112	26.8	3.4
4-pentenyl	370	isothiocyanate	127	23.6	3.8
2-hydroxy-3-butenyl-	371	oxazolidinethione	128	23.4	3.8
2-hydroxy-4-pentenyl-	385	oxazolidinethione	142	21.1	4.3
hydroxybenzyl-	408	acid	138	21.7	4.1
phenethyl-	405	isothiocyanate	162	18.5	4.9
3-methylthiobutyl-	412	isothiocyanate	169	17.4	5.1
4-methylthiopentyl-	426	isothiocyanate	183	16.4	5.5
indolyl-3-methyl-	432	acid	175	17.1	5.3
4-hydroxy-indolyl-3-methyl	448	acid	191	15.7	5.7
4-methoxy-indolyl-3-methyl	462	acid	205	14.6	6.2

[1] 1.1 mg/g butylisothiocyanate = 3.0 mg/g butylglucosinolate

3.0 mg/g butylisothiocyanate = 8.5 mg/g butylglucosinolate

The general problem of consistency in expression of analytical results for glucosinolates was addressed in 1980 at a meeting of rapeseed chemists from Europe and Canada which was held in Winnipeg, Manitoba. Daun A resolution from this meeting stated that

> "(1) Determination of the glucosinolate content of rapeseed meal and expression of resulted included when present allyl-, 3-butenyl-, 4-pentenyl-,2-hydroxy-3-butenyl-, and 4-hydroxybenylglucosinolates.
>
> (2) Results be expressed on the basis of oil-free air-dry meal.
>
> (3) It is preferable to express results in micromoles per g oil-free air-dry meal.
>
> (4) For purposes of trade, expression of results in milligram equivalents of 3-butenyl 3-butenyl glucosinolate per g of oil-free air-dry meal."

As a result of this agreement, almost all technical reports showing glucosinolate concentration now appear as micromoles per gram. This agreement was important because previously, glucosinolates had been reported variously as mg/g as butyl isothiocyanate, as mg/g (unspecified), as mg/g of other isothiocyanates, mg%, % and in other formats which were almost impossible to compare. the use of micromoles make it possible to compare components with large differences of molecular weight in terms of the same number of molecules, which are, from a physiological as well as chemical perspective, the active units.

The original canola definition also referred to an analytical method for determining glucosinolates, the "(GLC Method-MacGregor)", an unpublished method developed by Dr. D.I. MacGregor at Agriculture Canada's Saskatoon Research Station. Although this method was being used in various forms by several laboratories, its lack of publication and the lack of details about its performance was a serious problem, especially for newcomers to the canola industry. A major reason for including a reference to a specific method in the definition was to ensure that glucosinolates were not determined by one of the older methods which had been found to give erroneously low results.

In 1981, Drs. MacGregor (Saskatoon) and Daun (Winnipeg) were commissioned to prepare a method for determination of glucosinolates based on the best method then available. That method would be adopted by the Canadian industry as the official method for determining glucosinolates in canola. The method chosen was based on the recently published GLR method of Dr. Thies and Drs. Heaney and Fenwick. The method was written and tested in the Saskatoon and Winnipeg laboratories and published as a method of the Canadian Grain Commission. The method was revised in 1983 and again in 989 to correct minor errors and to include updated methodology. A collaborative study was carried out using Canadian laboratories and an on-going check sample program was set up by the POS Pilot Plant corporation.

In August, 1982 the canola definition was reworded so that subparagraph (a) of paragraph 5 read

> "(a) the oilseeds shall be the seed of the species Brassica napus or Brassica campestris the oil component of which seed contains less than 5% erucic acid and the solid component of which seed contains less than 30 micromoles of any one or any mixture of 3-butenyl glucosinolates, 4-pentenyl glucosinolate, 2-hydroxy-3-butenyl glucosinolate, and 2-hydroxy-4-pentenyl glucosinolate per gram of air dry, oil free solid (GLC Method of the Canadian Grain Commission)."

This definition incorporated the recommendations from the 1980 conference, restricting the definition to the sum of the 4 most common aliphatic glucosinolates present in canola. Sinalbin, or 4-hydroxybenzylglucosinolate was not included as this was only present through contamination of the canola seed with wild mustard or charlock (*Sinapis arvensis* L.).

A final change to subparagraph (a) was made on September 12, 1986 when the limit of 5% erucic acid was lowered to 2% erucic acid to reflect the requirements of GRAS status for the United States Food and Drug registration.

The canola definition was eventually adopted by Agriculture Canada in the Canada Agricultural Products Specifications Act (now withdrawn), the Feeds Act (dealing with specification and handling of animal feedstuffs) and the Seeds Act, dealing with specification and handling of seeds. Canola grades were established by the Canadian Grain Commission in 1986 but the grade specifications only required that canola be the seed of canola varieties referring indirectly to the seeds act. The definition also appears in product standards for canola oil and canola meal were also established under the Standards Council of Canada.

Since the establishment of the 1982 definition of canola (with its 1986 modification) there have been three major developments which suggested that the definition needed further updating. These developments included:

changes in our knowledge of which glucosinolates are present in canola and their relative importance;
the development of methodology with better accuracy and precision than the GLC method; and development of varieties of mustard seed, *Brassica juncea* L. with low levels of erucic acid and glucosinolates.

Even before the 1982 revision of the canola definition, it was well known that there were more than 4 glucosinolates present in canola seed (Table 2). The presence of phenethylglucosinolate and the methylthioalkylglucosinolates had been established as early as 1967. These components, although routinely determined in plant breeding programs, were not usually included in analytical summaries as they were present in very small amounts compared to the major aliphatic glucosinolates. The development of the gas chromatographic method for determining "intact" glucosinolates made possible the determination of both the hydroxy-alkyl and indolyl glucosinolates, the former of which had been originally determined spectrophotometrically as oxazolidinethiones.

Indolyl glucosinolates were a major addition to the list of glucosinolates known to be present in canola seed. Although first noted in canola seed by MacGregor in 1978, they were not included in the canola definition because

1) they were very difficult to determine with precision especially between laboratories (a situation which continues today);

2) the canola varieties produced up to 1982 appeared to have very similar levels of indolyl glucosinolates, breeding emphasis having been placed on the aliphatic group and;

3) there was little information available on the nutritional importance of this group of glucosinolates while considerable information had been gathered on the significant improvement of nutritional quality resulting from the reduction in aliphatic glucosinolates.

There continues to be controversy over the relative nutritional importance of various glucosinolates. It has generally been well established that the oxazolidinethiones formed on hydrolysis of hydroxyalkylglucosinolates are strongly goitrogenic, having an irreversible inhibitory effect on iodine uptake by the thyroid gland. Other glucosinolates which lead to formation of isothiocyanates may have goitrogenic or other antinutritional effects. Thiocyanate ion, formed on hydrolysis of hydroxyaromatic and indolyl glucosinolates has a reversible inhibitory effect on iodine uptake of the thyroid gland which can be controlled with additional dietary iodide. Indolyl glucosinolates, on the other hand have also been cited as possible anticarcinogenic compounds.

Continued development of canola type lines in other species would certainly require the expansion of the definition to include glucosinolates specific to those seed types such as sinigrin in mustard seed (*Brassica juncea*). The question of contamination from other brassica species (especially wild mustard which contains sinalbin also needed addressing.

Table 2. Glucosinolates Present in Canola

Glucosinolate	Typical Amount (μM/g, oil-free)	Typical Amount (μM/g, whole seed)
Major Glucosinolates		
3-butenyl-	1.2	0.6
4-pentenyl	0.4	0.2
2-hydroxy-3-butenyl-	7.6	4.2
2-hydroxy-4-pentenyl-	0.5	0.3
1-hydroxy-indolyl-3-methyl	6.5	3.6
Minor Glucosinolates		
allyl- [1]	0.2	0.1
hydroxybenzyl-[2]	1.1	0.5
phenethyl-	0.2	0.1
3-methylthiobutyl-	Tr.	Tr.
4-methylthiopentyl-	Tr.	Tr.
indolyl-3-methyl-	0.4	0.2
4-methoxy-indolyl-3-methyl	0.2	0.1
4-methylsulphinylbutyl-	0.4	0.2
4-methylsulphinyl-3-butenyl-	0.2	0.1
methyl	Tr.	Tr.
3-methysulphinylpropyl	Tr.	Tr.
3-methylthiopropyl	0.5	0.3

Analytical methodology for glucosinolate determination has also improved over the past few years. In particular, the HPLC methods adopted by ISO and AOCS allow for a greater degree of accuracy and precision and also offer the advantage of an internationally sanctioned methodology. In addition, several methods have been developed which allow relatively rapid and accurate determination of total glucosinolates, most commonly be determination of glucoses released on hydrolysis.

The 1980 resolution on reporting methods for glucosinolates, and the consequent canola definition called for reporting on an oil-free, air-dry basis. This reporting basis was chosen because

1. it was considered necessary to remove the oil from seed before proceeding with the analysis of glucosinolates, and

2. glucosinolates were considered a meal-related problem and it was thought desirable to report glucosinolates in the concentrations they would have in commercial meal.

Developments have shown both of these reasons to be inaccurate or fallacious.

Firstly, several studies have shown that glucosinolates can be analyzed without removing the oil from the seed. In the case of the "GLC Method of the Canadian Grain Commission", a collaborative study was carried out between the Grain Research Laboratory and the C.E.T.I.O.M. Laboratory in Orleans. Each laboratory analyzed samples of seed, ground seed and oil-free flour. No statistically significant different was noted between analyses of full-fat ground seed and oil-free

flour and it was concluded that the time-consuming oil removal step could be removed from the method. which it was in the 1989 edition.

Expression of seed glucosinolate contents on a fat-free basis simply because they are considered a meal related problem may be somewhat misleading. The canola definition expresses glucosinolates on the basis of oil-free dry material which is different from the meal residue remaining after commercial processing. Commercial processing results in the destruction of 30%-70% of the glucosinolates originally present in the seed (Table 3). Seed with glucosinolate levels well above the canola standard could thus be used to produce meals which meet the standard. Nutritional guidelines for utilization of canola meal were based on studies of meals made from seed with glucosinolate levels within the canola standard.

Table 3. Effect of processing on glucosinolates.

Crushing Plant	Samples		Glucosinolates µm/g, Oil-free				Destroyed in Processing	
	1979-81	1982-84	1979-81		1982-84		1979-81	1982-84
A	3	10	49	26	38	28	47	26
B	7	9	26	18	25	7	31	72
C	8	5	25	12	20	14	52	30
D	4	NS	22	7	NS	NS	68	
All	22	24	31	16	28	16	48	41

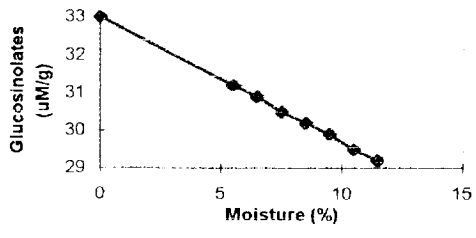

Figure 1. Effect of Moisture on Glucosinolates

The use of "air-dry" also leads to ambiguity in the reporting of glucosinolates. Depending on the ambient relative humidity, temperature and length of time exposed, the air-dry moisture of canola meals may range from 6% to 12%. Variation of moisture content during analysis of canola should not add additional error to the analysis. It is preferable to specify a constant moisture basis. At the Grain Research Laboratory, 8.5% moisture has been chosen as this is close to the long term moisture level in Canadian seed.

In 1992, the Canola Council of Canada reviewed the canola definition and proposed a revision to this definition to come into effect in 1997.

> "The oilseed shall be the seed of the genus *Brassica* which shall contain less than 18 micromoles of total glucosinolates per gram of whole seed at a moisture content of 8.5%; and the oil component of which seed shalls contain less than one percent of all fatty acids as erucic acid.
>
> Glucosinolates and erucic acid to be determined by the most recent ISO procedures.
>
> The term canola meal may be used to describe a protein meal derived from seeds of the genus Brassica. This product shall contain less than 30 micromoles of total glucosinolates per gram of meal at a moisture content o 8.5%
>
> Glucosinolates to be determined by the most recent ISO method.

> The term canola oil may be used to refer to oil derived from seeds of the genus *Brassica*, with less than 1% of fatty acids as erucic.
>
> Erucic acid to be determined by the most recent ISO methods." [4]

This definition took into account most of the problems described above and allowed for an increase in quality, both in terms of glucosinolates and erucic acid. The five year waiting period was established to allow the plant breeding community to adapt completely to the new definition. The rules of the canola recommending committee allowed new varieities to contain only a total of 12 micromoles per gram. Canadian canola, on average, already meets the proposed standards.

As the year 1997 approached, it appeared that adoption of the definition as it was proposed could create some problems. The United States is a major market for Canadian canola and it is important to retain GRAS status. This means that it is necessary to clear new oil types as they appear. Canola quality *Brassica juncea* is currently being cleared as GRAS and is expected to be commercially available in 1998 or 1999. The new definition would have to be adopted by at least two Canadian Acts of Parliament, the Feeds Act and the Seeds Act each change to these acts takes time and money. Additionally, Health Canada approval is required for any new oil types which might be considered novel.

In the spring of 1997, it was decided to proceed with a change in the definition using the following approach.

The canola definition will be changed to read

> The oilseed, oil and protein meal shall be derived from the species *B. napus,* B. rapa or B juncea and shall meet the erucic acid content maximum in the oil and glucosinolate content maxima in the seed and protein meal as specified in standards registered with the Canadian General Standards Board (CGSB) and as determined by the latest officially accepted International Standards Organization Methods.

It is felt that this change would allow the greatest flexibility in application of the definition since the CGSB Standards can be adapted to meet commercial reality and are subject to review at least every five years.

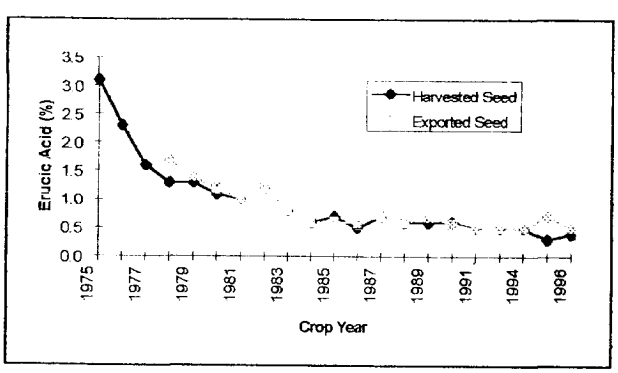

Figure 2a. Erucic Acid Levels of Canadian Canola

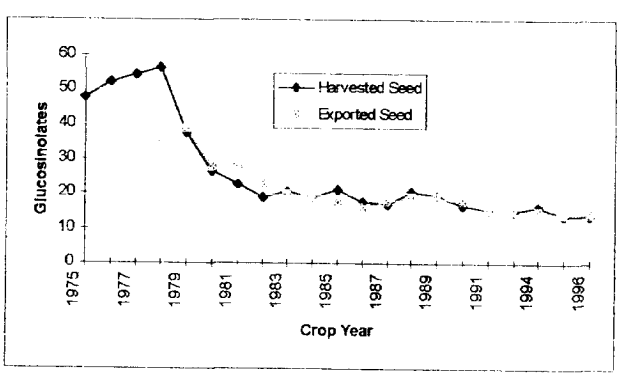

Figure 2b. Glucosinolates in Canadian Canola

As adoption of the new canola definition proceeds, there are several issues which must be considered. Firstly, the first lines of the new *B. juncea* varieties with low levels of glucosinolates and erucic acid may not quite meet the guidelines specified by the Registration Recommending

Committee although they will be within the levels specified in the standard. This will require some flexibility in registration. The first new *B. juncea* varieties may also have slightly different fatty acid compositions than traditional canola and some temporary adjustments may be required.

While developing the standards which will be used to define canola, it will also be necessary to consider the role of oils with modified fatty acid composition, especially those which depart from the generally desired characteristics of low saturated fatty acids. How will they fit in?

Although the new canola definition will reflect today's commercial reality, there will doubtless be challenges ahead.

Reference List

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