Extent of Thermal Decomposition of Indole Glucosinolates During the Processing of Canola Seed

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A series of samples representing various stages of canola seed processing were obtained from two commercial processing plants to assess the extent of thermal degradation of indole glucosinolates during the crushing process. The individual glucosinolate content of all samples was determined along with the content of indole glucosinolate thermal degradation products [indoleacetonitriles and thiocyanate ion (SCN)]. Only minor decomposition of indole glucosinolates was evident prior to the desolventization stage of seed processing. Major decomposition of indole glucosinolates occurred in the desolventization of seed processing with little or no effect during meal drying. Indoleacetonitriles (3-indoleacetonitrile and 4-hydroxy-3-indoleacetonitrile) and SCN together accounted for 45-60% of the degraded indoles in the samples studied.

Indole glucosinolates which represent a significant proportion of the total glucosinolate content of canola (low-glucosinolate rapeseed) meal (1) have been shown to be susceptible to thermal degradation (2). Thiocyanate ion (SCN) has been reported as a major thermal degradation product of indole glucosinolates (1,3) and, recently, indoleacetonitriles (3-indoleacetonitrile and 4-hydroxy-3-indoleacetonitrile) were identified as additional thermal degradation products (4).

Heat treatment is applied in commercial rapeseed processing plants to condition the seed for the improvement of oil extraction, to inactivate myrosinase enzyme and for solvent removal and drying of meal (5). The conditions (time, temperature, moisture, etc.) involved at these various stages of the commercial crushing process may be sufficient to effect thermal decomposition of indole glucosinolate and, in this regard, the nutritive quality of the meal produced may be affected. The current study was undertaken to assess the extent of thermal degradation of indole glucosinolates in the commercial crushing of canola and to quantify the production of SCN and indoleacetonitriles at various stages of the crushing process.

MATERIALS AND METHODS

A series of samples representing various stages of canola seed processing were obtained from two commercial crushing plants (A and B) located in Western Canada. The samples obtained included: I, seed; II, flakes; III, cooked flakes; IV, expelled cake; V, extracted meal; VI, desolventized meal; VII, dried meal. Only samples I, IV and VII were obtained from crushing plant A, whereas all samples were collected at crushing plant B. Duplicate samples were collected at crushing plant A on two consecutive days, and the sampling was conducted at the various stages of processing at a time interval corresponding to the flow of seed through the crushing plant. In crushing plant B, duplicate samples were collected at all stages (I-VII) of processing and the collections were conducted on the same day with approximately 30 min between duplication collections at each stage (i.e., no attempt was made to follow seed through the process but rather, a homogenous batch of seed being processed was assumed). Moisture content was determined for all samples which were then freeze-dried, ground and defatted with hexane prior to analysis for glucosinolates, SCN and indoleacetonitriles.

Individual glucosinolates were determined by gas liquid chromatography (GLC) as desulphotrimethylsilyl (TMS) derivatives according to the method of Slominski and Campbell (1). In the GLC determination, relative response factors (RRF) were calculated from the ratios of benzyl glucosinolate (internal standard) TMS carbon number and the respective glucosinolate TMS carbon number. Variations in flame ionization detector response caused by the different chemical structures of the various glucosinolates were accounted for by the use of correction factors of 0.72 for aliphatic glucosinolates, 1.00 for 4-hydroxy glucosinolate and 1.48 for indole glucosinolates. Pure samples of allyl glucosinolate (Aldrich) and benzyl glucosinolate (Canola Council of Canada) and a sample of 4-hydroxy-3-indoylmethyl glucosinolate isolated from canola seed according to the method of Slominski and Campbell (1) were used in the determination of the correction factors. A GLC method developed by Slominski and Campbell (6) was used for the determinations of 3-indoleacetonitrile and 4-hydroxy-3-indoleacetonitrile. The method of Johnson and Jones, as modified by Slominski and Campbell (1) was used for the determination of SCN. Sample variation was assessed according to standard statistical procedures (7).

RESULTS AND DISCUSSION

The total glucosinolate content of the canola seed samples from crushing plants A and B is shown in Table 1. It

TABLE 1

Glucosinolate Content of Canola Seed Processed at Two Commercial Crushing Plants (μmol g^{-1}, Oil Free Meal)

	Crushing plant	
Glucosinolate	A	B
Allyl	1.68	n.d.[a]
3-Butenyl	6.13	3.32
4-Pentenyl	3.04	0.45
2-Hydroxy-3-butenyl	12.40	6.96
2-Hydroxy-4-pentenyl	1.28	0.16
4-Hydroxy-benzyl	1.58	3.35
3-Indolylmethyl	0.51	1.04
4-Hydroxy-3-indolylmethyl	8.47	8.56
Total ± SD	35.09 ± 1.71	23.84 ± 0.64

[a]Not detected.

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can be suggested from the profile of aliphatic glucosinolate present in the two seed samples that *B. napus* seed predominated in crushing plant B, while the seed in crushing plant A was probably of the *B. campestris* type. Both samples revealed a similar level of indole glucosinolates. Admixtures of weed seeds such as commerical mustard, wild mustard and stinkweed were indicated by the contents of allyl and 4-hydroxybenzyl glucosinolates in the samples. Although the extent of weed seed contamination varied between crushing plants, the data are similar to that noted for canola meals analyzed previously in this laboratory (Slominski and Campbell, unpublished results).

The data for moisture contents of the canola seed and processed seed at various stages of processing indicated low values (5.0-7.5%) with no increase in moisture prior to the desolventizer stage of processing. These data are consistent with the general practice followed by crushing firms in Western Canada where live steam is not added to flakes (preexpeller cooking) during the process, but relatively mild, dry heat treatment is employed at this stage of processing to effect the production of oil with a low sulfur content (5).

The relatively constant level of aliphatic glucosinolates evident in the samples of processed seed for all stages prior to the desolventizer stage indicates that little or no autolysis of glucosinolates occurred (Fig. 1). This result would be expected due to the relatively low moisture contents of the samples since Youngs and Wetter (8) have demonstrated that hydrolysis of glucosinolates by myrosinase is minimal unless the moisture content exceeds 10%. In this regard, Slominski *et al.* (9) have demonstrated that during the crushing process myrosinase may remain active up to the desolventizer stage. In contrast to the data for aliphatic glucosinolates, the level of indole glucosinolates tended to be lower for cooked flakes and expelled cake than for seed (Fig. 1). Since indole glucosinolates are more susceptible then aliphatic glucosinolates to heat treatment (1) this decrease may be a reflection of thermal decomposition of the indole glucosinolates. The data for SCN contents of these fractions (Fig. 2) also is indicative of some thermal decomposition of indole glucosinolates.

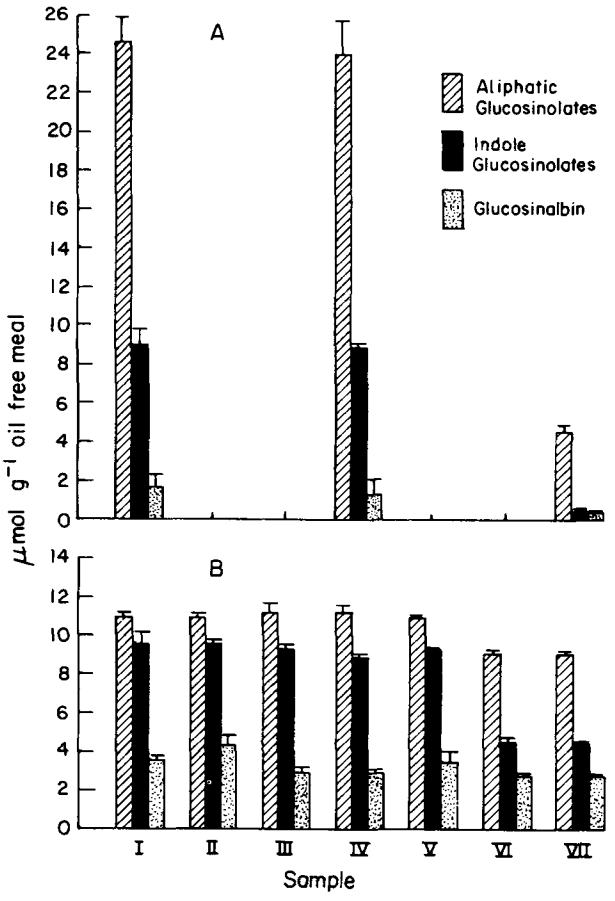

FIG. 1. Intact glucosinolate content of canola seed at various stages of processing in two commercial crushing plants (A and B); I, seed; II, flakes; III, cooked flakes; IV, expelled cake; V, extracted meal; VI, desolventized meal; VII, dried meal.

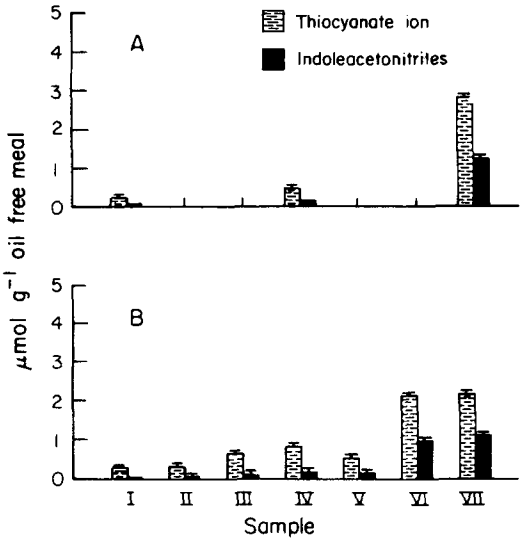

FIG. 2. Thiocyanate ion and indoleacetonitrile contents of canola seed at various stages of processing in two commercial crushing plants (A and B). See Figure 1 for details regarding stages.

In crushing plant B, a relatively high content of indole glucosinolates was present in extracted meal as compared with expelled cake (Fig. 1). The reason for this difference is not clear, but it may be related to variability in the sampling procedure since the same batch of seed was not followed through the process. Glucosinalbin showed greater variability than other glucosinolates, which may also reflect the sampling procedure. Glucosinalbin is present in the samples because of weed seed contamination and, consequently, might be expected to show high variability from one batch of seed to another. An influence of the sampling procedure on glucosinolate concentrations is also indicated by the fact that, in general, greater variability between duplicate samples was apparent when samples were collected on separate days (plant A) as opposed to being collected on the same day with only a short time lapse between samplings (plant B) (Fig. 1).

Desolventized meal in crushing plant B showed a decrease in glucosinolate content in comparison to extracted meal, and the effect was most marked for indole glucosinolates. The decrease was probably due to thermal decomposition of the glucosinolates which

would be favored by the relatively high moisture content (13%) and high final temperature (105°C) that existed in the desolventizer. In laboratory trials, Slominski and Campbell (1) demonstrated that little or no decomposition of glucosinolates occurred due to dry heat treatment (100°C), while substantial breakdown was caused by wet heat treatment (100°C), especially for indole glucosinolates. In the current study it is unlikely, despite the moisture content of the desolventized meal, that hydrolysis of glucosinolates by myrosinase contributed to the decrease in glucosinolate content as the relatively high temperature of the meal in the desolventizer would have resulted in the complete inactivation of myrosinase. Drying of the meal in crushing plant B did not result in any further decrease in the glucosinolate content of the meal (Fig. 1). In this regard, the marked decrease in glucosinolate content that was evident for dried meal from crushing plant A was probably a consequence of thermal decomposition in the desolventizer (Fig. 1). The greater destruction of glucosinolates in plant A as compared to plant B may have been due to the greater residence time of meal in the desolventizers (180 vs 30 min) as the final temperatures (105-106°C) in the desolventizer were similar for the two plants.

A comparison of Figures 1 and 2 indicates that the contents of indoleacetonitriles in the various samples for both crushing plants A and B followed the extent of decomposition of indole glucosinolates. In general, this was also the situation for SCN. Glucosinalbin, however, is also a potential source of SCN, and, consequently, variability in glucosinalbin content among samples may have masked any relationship between indole glucosinolates and SCN. Indole glucosinolate appeared to be the major source of SCN in the samples from crushing plant B, but due to the greater degree of decomposition of glucosinolates in crushing plant A this same relationship, while possible, was not entirely evident.

The data presented in Table 2 show a comparison of the amounts of decomposed indole glucosinolates in relation to indoleacetonitrile production and release of SCN. Of the total for indole glucosinolate decomposition (8.7 and 5.3 μmol g^{-1} in crushing plants A and B, respectively) approximately 45-60% was accounted for in the meal as a combination of indoleacetonitriles and SCN. These data are in agreement with previous results (6) and, consequently, the production of as yet unidentified breakdown products of indole gluconsinolates is indicated during the desolventizing stage of processing in commercial crushing plants. The presence in meal of some such product(s) which releases SCN upon hydrolysis in the GI tract of poultry has been demonstrated (10).

TABLE 2

Comparison of the Decomposition of Indole Glucosinolates with Indoacetonitrile and Thiocyanate Ion Production During the Processing of Canola Seed (μmol g^{-1})

	Crushing plant[a]	
	A	B
Indole glucosinolate decomposed[b]		
3-Indolylmethyl	0.4	0.3
4-Hydroxy-3-indolylmethyl	8.3	5.0
Total	8.7	5.3
Indoleacetonitrile and SCN production[c]		
3-Indoleacetonitrile	0.2	0.2
4-Hydroxy-3-indoleacetonitrile	1.0	0.9
Thiocyanate ion (SCN)	2.7	2.1
Total	3.9	3.2

[a]Samples were obtained from two commercial crushing plants (A + B).
[b]Difference between seed (stage I) and dried meal (stage VII) values.
[c]Dried meal (stage VII) values.

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