DETOXIFICATION OF *BRASSICA JUNCEA* WITH AMMONIA

D. I. McGregor
Agriculture Canada Research Station
Saskatoon, Saskatchewan, Canada
J. A. Blake
POS Pilot Plant, Saskatoon, Saskatchewan, Canada
M. D. Pickard
CSP Foods, Saskatoon, Saskatchewan, Canada

Conversion of rapeseed production to low erucic acid varieties has drawn attention to the continuing need of high erucic acid oil for industrial purposes. In Western Canada high erucic acid varieties of rapeseed are currently grown under contract to meet this need. Although it is recognized that brown or Oriental (yellow seeded) mustard (*B. juncea* [L.] Coss.) has a greater potential as a high erucic acid crop, production is precluded by the presence of glucosinolates which can cause palatibility problems or be toxic when oil-extracted meal is used in animal feeds. Recently sources of low erucic acid have been isolated from *B. juncea* mustard (1), increasing the potential for production as an edible oilseed crop. However, glucosinolates must still be removed for this potential to be fully realized.

Plant breeding is the most economical means of removing glucosinolates in the long term. To this end a notable achievement has been made in the development of low glucosinolate varieties of rapeseed. Attempts to reduce the glucosinolate content of *B. juncea* mustard have not yet met with the same success.

An alternate approach to removing the glucosinolates is base catalyzed degradation. Kirk and co-workers (2) removed the 2-hydroxy-3-butenyl glucosinolate from crambe (*Crambe abyssinica* ex R. E. Fries) using an ammonia-heat treatment and suggested the treatment could be a commercially feasible means of debittering and detoxifying both crambe and rapeseed (*B. campestris* L. and *B. napus* L.) (3). More recently Friis and co-workers (4) observed that allyl glucosinolate, the major glucosinolate of *B. juncea* mustard seed, was degraded rapidly and nearly quantitatively to vinylglycine (2-aminobut-3-enoic acid), thioglucose and sulphate when incubated at ambient temperature with sodium hydroxide. Other glucosinolates were observed to undergo base catalyzed degradation but not as readily and with considerable side reactions which gave rise to

nitriles.

The relative ease with which allyl glucosinolate may be degraded was substantiated in the present study by treatment of Cruciferous seed of varying glucosinolate composition with ammonium hydroxide. A 3 g sample of seed was placed in a 3 ml hypo vial with 0.3 ml concentrated (28 %) ammonium hydroxide, the vial sealed with a silicone rubber septum and let stand at ambient temperature for 1 week. The seed was then air-dried overnight, oil extracted by Swedish tube (5) using petroleum ether (b. p. 30-60 C), and glucosinolate content determined essentially by the method of Underhill and Kirkland (6) but using trehalose as internal standard and derivatizing for only two hours.

The allyl glucosinolate content of both B. juncea var. Lethbridge 22A and Thlaspi arvense L. stinkweed was reduced by more than 90 % as a result of the ammonium hydroxide treatment (Table 1). The glucosinolate content of B. napus var. Midas was reduced by 14 %. This was due to a drop in the content of 2-hydroxy-3-butenyl glucosinolate, 3-butenyl- and 4-pentenyl glucosinolates having remained unreduced. The 3-butenyl glucosinolate content of B. campestris var. R-500 also remained unchanged as did the 4-hydroxybenzyl glucosinolate of B. hirta Moench var. Gisilba. The results suggest that treatment with ammonia or ammonium hydroxide would debitter and detoxify more readily when applied to allyl glucosinolate of B. juncea mustard than when applied to the 2-hydroxy-3-butenyl glucosinolate of crambe or the 3-butenyl-, 4-pentenyl- and 2-hydroxy-3-butenyl glucosinolates of rapeseed.

To assess the commercial feasibility of detoxifying B. juncea mustard the rate and duration of heating of a commercial desolventizing-toasting process was simulated on a laboratory scale using B. juncea var. Blaze. To ensure that glucosinolate breakdown could not occur by myrosinase activity, the enzyme was inactivated by conditioning the seed to 10 % moisture then heating in boiling water for 5 minutes. Following drying in a forced-air oven at 45 C for 16 hours 300 g lots of seed were ground in a coffee mill and extracted for 8 hours with petroleum ether. The solvent was filtered off under suction and the meal air-dried. Oil-extracted meal, 150 g, was placed in a cylindrical cooker measuring 10 cm in diameter by 19 cm in length. Water or water plus ammonium hydroxide was added while the meal was being mixed in the cooker with a paddle wheel type mixer consisting of two blades mounted on a shaft running the

length of the cooker and which was driven by an externally mounted motor at 75 rpm. The circumference of the cooker was fitted with a rheostatically controlled heating mantle and thermocouple. Heating was commenced at 57 C for 3 minutes, increased 3 C per minute to 95 C, then 1 C every 2 minutes to 110 C and held for 3 minutes to simulate a commercial desolventizing-toasting process.

Allyl glucosinolate content was determined by the modified method of Underhill and Kirkland (6). Sinapine was determined by the method of Tzagoloff (7). Total nitrogen was determined by microkjeldahl digestion followed by colorimetric measurement (8). Non-protein nitrogen was determined by first adding 30 ml water to a 2 g sample, mixing for 1 hour, then centrifuging. To 3 ml of the supernatant was added 15 ml of absolute ethanol. After standing 10 minutes the sample was centrifuged and the supernatant digested.

Initial studies indicated that an ammonia concentration of 1.5-2 % in combination with a moisture content of ca 20 % were required to achieve the maximum rate of degradation. Subsequent studies were carried out with an ammonia concentration of ca 2 % and/or a moisture content of 20-25 %. Following addition of water only and heating, allyl glucosinolate content was reduced by 22.8 % indicating that appreciable thermal decomposition had occurred (Table 2). Addition of water plus ammonium hydroxide and heating resulted in an even greater reduction of 94.2 %. The residual allyl glucosinolate content of 6.9 umoles per g oil-extracted moisture-free meal was well within the range of alkenyl glucosinolates (< 30 umoles) designated as canola rapeseed and therefore could be expected to improvement meal quality.

A further potential improvement in the quality of the product was the reduction of sinapine by 74 %. Although less than the 90 % reduction observed in similar studies with Crambe by Kirk and co-workers (2), these researchers found that aqueous ammonia was not as effective as gaseous ammonia in reducing sinapine.

Increase in the total nitrogen content of 0.8 g per 100 g oil-extracted moisture-free meal represented ca 34 % of the nitrogen added as ammonium hydroxide. This increase was of the same order of magnitude as previously observed with Crambe (2). Nitrogen added to feedstuffs as a result of ammoniation has been shown to be utilized by ruminant animals (9, 10).

Studies with Crambe have shown that ammonia may be retained in two forms: as weakly bound ammonia sorbed to protein and as strongly bound ammonia most likely reacted

with glucosinolates, sinapine, reducing sugars and protein (2). Although the nature of the bound ammonia was not thoroughly investigated it appeared that about half was extractable as non-protein nitrogen.

Commercial feasibility of detoxifying *B. juncea* mustard with ammonia was further assessed by processing the variety Lethbridge 22A on a pilot plant scale at the POS Pilot Plant in Saskatoon. Seed was conditioned to 9.5 % moisture in a Ribbon blender, flaked with Turner Ipswich 46 cm dia. chilled cast iron rolls, cooked and expelled with a Simon Rosedowns laboratory screw press with two 46 cm dia. by 36 cm deep trays, extracted with a Crown Iron Works 13 by 20 cm bed cross section percolation solvent extractor and meal desolventized in a two tray 76 cm dia. desolventizer equipped with steam injectors in the sweep arms in each tray and a water spray in the top tray. In place of simple steam stripping in the top tray a combination of steam, water and ammonia was added. Solvent, water and excess ammonia were stripped out in the bottom tray. Three processing runs were made one of 633 kg and two of 1048 kg seed. The operating parameters for the three runs ranged as follows:

Primary		Flammable	
Seed moisture %	9.2-9.5	Ext. feed rate kg/hr	73.3-79.6
Flaking rate kg/hr	118-124	Meal output kg/hr	67.0-79.4
Flake mm	0.26-0.35	Oil output kg/hr	7.8-10.1
Cooker top tray C	58-66	NH_3 rate, % feed rate	6.0- 8.2
Cooker bottom C	90-98	Water rate kg/hr	9.1-12.5
Cake prod. kg/hr	80-86.4	Steam rate kg/hr	2.4- 3.7
Oil prod. kg/hr	33.7-35.1		

Glucosinolate content was determined by the method of Heaney and Fenwick (11) using benzyl glucosinolate as internal standard. Nitriles were determined, after extraction into methylene chloride, by gas chromatography using a FFAP column.

Initial glucosinolate content of 200 umoles per g oil-extracted air-dry meal was reduced to 60-100 umoles in the top tray of the DT and to 13-24 umoles after exiting the bottom tray. Analysis of the meal, stack gas and hexane in the solvent work tank indicated little (1.9-5.6 %) conversion to nitriles.

As with the laboratory scale study excess ammonia was added to reduce the glucosinolate content. It appeared that most of the excess ammonia was absorbed into the water in the solvent work tank. During the

latter two runs the level climbed steadily reaching as high as 2.2 %. An odour of ammonia was present in the Flammable. This was in part derived from leaks in some lines but also from the reboiler once the concentration of ammonia became high in the solvent work tank water. Meal in the top tray of the DT did not smell strongly of ammonia even at 70 C.

With appropriate recycling of the excess ammonia, detoxification of B. juncea mustard meal during desolventizing-toasting appears commercially feasible (12).

Able assistance of J. L. Lagimodiere is appreciated. The laboratory cooker was kindly loaned by C. G. Youngs. Financial support was provided by the Market Development Fund of the Saskatchewan Department of Agriculture.

(1) Kirk, J. T. O. & Oram, R. N. (1981) J. Aust. Inst. Sci. 47,51-52
(2) Kirk, L. D., Mustakas, G. C. & Griffin Jr., E. L. (1966) J. Amer. Oil Chem. Soc. 43,550-555
(3) Mustakas, G. C. & Kirk, L. D. (1968) U. S. Patent 3,392,026
(4) Friis, P., Larsen, P. O. & Olsen, C. E. (1977) J. Chem. Soc. Perkin Trans. 1,661-665
(5) Troeng, S. (1955) J. Amer. Oil Chem. Soc. 32,124-126
(6) Underhill, E. W. & Kirkland, D. F. (1971) J. Chromatog. 57,47-54
(7) Tzagoloff, A. (1963) Plant Physiol. 38,202-206
(8) Stringam, G. R., McGregor, D. I. & Pawlowski, S. H. (1974) Proc. 4th Int. Rapeseed Conf. Giessen, West Germany 99-108
(9) Waiss, A. C., Guggolz, J., Kohler, G. O., Walker Jr., H. G. & Garrett, W. N. (1972) J. Anim. sci. 35,109-122
(10) Walker Jr., H. G., Kohler, G. L., Graham, R. P., Hart, J. R. & Garrett, W. N. (1976) U. S. Agric. Res. Serv. Cent. Reg. (Rep.) 105-121
(11) Heaney, R. K. & Fenwick, G. R. (1980) J. Sci. Food Agric. 31,539-599
(12) Canada Patent Appl. 417,759; U. S. Patent Appl. 449,179; Great Britain Patent Appl. 8,236,125; Australian Patent Appl. 91495/82

Table 1. Effects of treating Cruciferous seed of varying glucosinolate composition with ammonium hydroxide on the glucosinolate content.

Species and Variety	Untreated (umoles/g oil-extracted moisture-free meal)						Ammonia treated (umoles/g oil-extracted moisture-free meal)					
	All	But	Pent	HO But	HO Benz	Total	All	But	Pent	HO But	HO Benz	Total
B. juncea Leth. 22A												**
$\bar{x}$(a)	129.8	2.7	-	0.9	-	133.5	7.7	3.5	-	0.4	-	11.6
S.D.	4.4	2.0		0.3		3.9	1.6	1.6		0.3		2.6
T. arvense Stinkweed												**
$\bar{x}$	121.4	0.1	-	-	-	121.5	6.1	2.6	-	0.2	-	8.9
S.D.	0.9	0.1				1.0	0.6	0.2		0.1		0.8
B. napus Midas												*
$\bar{x}$	1.7	22.4	4.7	71.4	-	100.2	-	22.6	4.4	58.8	-	85.8
S.D.	0.2	0.7	0.4	3.5		4.7		1.6	0.3	3.7		5.6
B. campestris R-500												NS
$\bar{x}$	-	153.4	0.9	2.2	-	156.4	-	142.1	0.7	1.7	-	144.6
S.D.		7.5	0.3	2.5		7.4		5.1	0.3	2.3		7.5
B. hirta Gisilba												NS
$\bar{x}$	5.0	-	-	2.9	161.8	169.6	-	-	-	3.0	166.6	169.6
S.D.	4.0			0.3	15.4	12.7				0.8	1.4	1.5

(a) Mean and standard deviation of three replicates.
* Significant at the 5% level within sample.
** Significant at the 1% level within sample.

Table 2. Effect of treating *B. juncea* var. Blaze meal with water and/or ammonium hydroxide to obtain ca 25 % moisture and ca 2 % ammonia by weight followed by heating to simulate a desolventizing-toasting process on allyl glucosinolate, sinapine, total and non-protein nitrogen content.

Treatment: NH_4OH (ml / 100 g meal)	Treatment: H_2O (ml / 100 g meal)	Treatment: Heating		Allyl Glucosinolate (umoles / g oil-extracted moisture-free meal)	Sinapine (umoles / g oil-extracted moisture-free meal)	Total nitrogen (g / 100 g oil-extracted moisture-free meal)	Non-protein nitrogen (g / 100 g oil-extracted moisture-free meal)
0	0 (a)	-	$\bar{x}$ (b)	119.2	1.89	7.9	0.4
			S.D.	14.9	0.11	0.3	0.1
0	28.8	+	$\bar{x}$	92.0	1.37	7.5	0.3
			S.D.	1.7	0.04	0.7	0.1
20	6.5	+	$\bar{x}$	6.9	0.49	8.7	0.9
			S.D.	6.0	0.01	0.3	0.1

(a) Moisture content of the meal 7.6 %.
(b) Mean and standard deviation of three replicates.