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Effect of Methanol–Ammonia–Water Treatment on the Fate of Glucosinolates

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Canola seed, Altex variety, was treated with 10% ammonia in a 95% methanol–hexane extraction system under forcing conditions. The four specific aliphatic glucosinolates, namely gluconapin, progoitrin, glucobrassicanapin, and gluconapoleiferin, for which canola seed is generally analyzed were extracted, mainly in the intact form, into the polar phase. Nearly 10% of the total aliphatic glucosinolates was retained in the meal. Almost 19% of the original glucosinolates was degraded in this process, and these were predominantly extracted into the polar phase. Two isolated glucosinolates, namely progoitrin and gluconapin, were also subjected to this process in model experiments. The breakdown products of aliphatic glucosinolates were nitriles, hydroxy nitriles, epithio nitriles, and isothiocyanates. No oxazolidinethione was produced in this process.

Rapeseed could play an important role in the world's protein supplies due to its climatic adaptability and the excellent nutritional value of its protein. However, the usefulness of rapeseed as a source of food protein is limited by the presence of undesirable components such as glucosinolates, phytates, phenols, and fiber (hull). Glucosinolates, the principal antinutrients found in rapeseed, give rise to hydrolysates possessing goitrogenic and toxic properties (Fenwick et al., 1983). Canadian rapeseed varieties, canola, low in both glucosinolates (<30 μmol/g of meal) and erucic acid (<2% in oil) content, have been available for over a decade. Nonetheless, even these glucosinolate levels are still too high for inclusion of canola meal into food products. Extensive work on the detoxification of rapeseed meal with respect to glucosinolates and/or their breakdown products has been reported (Rutkowski, 1970; Afzalpurkar et al., 1974; Maheshwari et al., 1981). However, commercial application of these methods is not feasible due to high processing costs, high loss of proteins, poor functional properties of the resultant products, and incomplete removal of the glucosinolate degradation products. Thermal inactivation of endogeneous myrosinase, which is responsible for glucosinolate hydrolysis, is the current commercial practice (Eapen et al., 1968). The intact glucosinolates left in the meal are, however, still capable of inducing undesirable effects by forming toxic aglycons in the gastrointestinal tract. Oginsky et al. (1965) have shown that some bacteria, particularly *Para colobatrum*, common to the digestive system of man, have myrosinase activity. Recently, a new process for the removal of glucosinolates from canola and from Midas rapeseed and mustard seed was developed (Rubin et al., 1984; Naczk et al., 1986, 1988; Shahidi et al., 1988). Crushed seeds were treated with a two-phase solvent extraction system. The first phase was 95% methanol containing 10% ammonia, and hexane, which

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extracted the oil, was the second phase. An improved canola meal with total aliphatic glucosinolates content below the detection limit of Wetter and Youngs method (1976) was obtained. Although this method was suitable during the development of the process, its limitations were fully realized. In particular, indole glucosinolates, which may constitute up to 50% of the total glucosinolates in canola, were not determined by this method. Thus, more accurate GC and/or HPLC methods were employed (Shahidi and Gabon, 1988). Furthermore, the fate of glucosinolates in this process had to be determined.

In this paper, the effect of CH_3OH–NH_3–H_2O treatment on the concentration of individual glucosinolates in Altex variety of canola and the fate of aliphatic glucosinolates in the seed as well as of two isolated glucosinolates in model systems is reported.

MATERIALS AND METHODS

Canola seed (Altex variety) was obtained from POS Pilot Plant Corp., Saskatoon, Saskatchewan. Sinigrin was purchased from Aldrich Chemical Co., and glucotropaeolin was acquired through the Canola Council of Canada, Winnipeg, Manitoba. Gluconapin and progoitrin were supplied by Dr. G. R. Fenwick, AFRC Food Research Institute, Norwich, U.K.

The derivatizing reagents, *N*-methyl-*N*-(trimethylsilyl)-trifluoroacetamide (MSTFA) and trimethylchlorosilane (TMCS), and pyridine (silylation grade) were obtained from Pierce Chemical Co., Rockford, IL. Other chemicals were purchased from Sigma Chemical Co., St. Louis, MO.

Hexane-extracted Altex meal was prepared by grinding the seeds in a Phillips coffee mill and extracting with hexane for 24 h on a Soxhlet apparatus. The defatted meal was dried at 40 °C in a vacuum oven. Methanol–ammonia–water-treated meal was prepared by the two-phase solvent extraction system as described below.

Ground Altex seed (30 g) was blended at low speed (approximately 15 000 rpm) in a Waring blender and placed in a fume hood to prevent vapor accumulation for 2 min with 200 mL of 95% methanol containing 10% ammonia. After a 15-min quiescent period, 200 mL of hexane was added and the mixture was blended again for 2 min. The meal containing residual oil was separated by vacuum filtration using Whatman No. 41 filter paper, rinsed three times with 100-mL portions of methanol, and dried at 40 °C in a vacuum oven. Residual oil in the cake was removed with hexane on a Soxhlet apparatus and again dried as before. The two-phase solution was separated with use of a separatory funnel. Additional oil was extracted from the polar phase with three 100-mL portions of hexane. Extracted oil and gums (solids in the polar phase) were recovered by evaporation of the solvent under vacuum.

Solutions of pure glucosinolates, namely progoitrin and gluconapin (1.0 μmol/mL), in 95% methanol containing 10% ammonia were heated at 46 °C for 2 min, twice, with a 15-min interval in between (to simulate the conditions during the blending process) and then were allowed to stand at room temperature for 24 h. Aliquots (0.5 mL) were analyzed for the sugar-related and aglycon degradation products. The intensity of the absorbance at 227 nm was used to determine the percentage of glucosinolate decomposition after the treatment.

The glucosinolate content of the meal was determined by gas chromatography using the method of the Canadian Grain Commission as developed by Heaney and Fenwick (1980) and modified by Daun and McGregor (1981), with further slight modifications (Shahidi and Gabon, 1988).

TMS derivatives of desulfoglucosinolates were separated on a fused silica SPB-20 capillary column (30 m × 0.25 mm (i.d.)) using a Hewlett-Packard gas chromatograph Model 5890A equipped with flame ionization detector and a 3392A Hewlett-Packard area integrator. Helium was used as the carrier gas.

The separation of underivatized desulfoglucosinolates was achieved on a Hewlett-Packard ODS-Hypersil (C_{18}) reversed-phase column (5-m packing; 200 mm × 4.6 mm (i.d.)) using a HP 1090A high-performance liquid chromatograph coupled to a diode array detector at a wavelength setting of 227.5 mm (Minchinton et al., 1982). A two-component solvent system consisting of water and acetonitrile was employed (Shahidi and Gabon, 1988). The identity of desulfoglucosinolates was confirmed by electron impact (EI) and ammonia chemical ionization (CI) mass spectra of their TMS derivatives and by comparison with the literature spectra.

Determination of decomposition products of glucosinolates was carried out as given below. Glucosinolate solutions (0.5-mL aliquots) were removed and transferred to 1-mL GC vials for the analysis of sugar-related breakdown products. The volume of the solvent in the GC vial was reduced to approximately 0.1 mL. Then, 0.5 mL of distilled water and either another 0.5 mL of distilled water or internal standard (0.5 mL; 0.5 μmol/mL of β-D-glucose in distilled water) were added. This mixture was applied to a microcolumn of DEAE-Sephadex A-25, as described elsewhere (Shahidi and Gabon, 1988), and the eluate was collected in a 3-mL vial along with 0.5 mL of distilled water washings from the column. The solvent was removed under reduced pressure, and the residue was further dried under a stream of nitrogen. The sample was derivatized and separated by GC. Concentrations of sugar-related breakdown products were calculated relative to the internal standard, glucose, and with respect to their individual TMS-derivatized carbon numbers.

Determination of aglycon decomposition products and furfuryl alcohol was carried out as follows. Internal standard (1 μL; 0.140 μmol/mL of heptyl isothiocyanate in methanol) was added to the aliquot (0.5 mL). The volume of the solvent in the GC vial was carefully reduced to about 0.1 mL. Dichloromethane (0.5 mL) was added to the vial, along with two glass beads. The vial was capped and was shaken for 2 h with use of a wrist action Burrell shaker at maximum speed. The layers were separated, and 250 μL of the dichloromethane layer was transferred into another 1-mL GC vial and dried over anhydrous sodium sulfate. The extract was separated on fused silica SPB-20 (30 m × 0.25 mm (i.d.)) or fused silica Supelcowax 10 (30 m × 0.25 mm (i.d.)) capillary column by the instrumentation and gas flow rates as described elsewhere (Shahidi and Gabon, 1989). The separated compounds were identified by comparison of their retention times with those of authentic samples and/or by mass spectrophotometric analysis.

Table I. Mass Balance and Protein Content of Altex Canola As Affected by Solvent Extraction[a]

solvent	meal, oil-free, %	oil, %	gums, %	protein in meal (N × 6.25), %
hexane	53.3 ± 0.3	46.9 ± 0.2		39.4 ± 0.3
CH_3OH–NH_3–H_2O hexane	44.3 ± 0.1	46.5 ± 0.7	7.8 ± 0.2	49.8 ± 0.8

[a] Values are, on dry-basis, mean values of five determinations ± standard deviation. [b] Low molecular weight polar compounds dissolved in methanol phase are referred to as "gums".

RESULTS AND DISCUSSION

Table I summarizes the mass balance and protein content of Altex canola as affected by CH_3OH–NH_3–H_2O hexane extraction. The yield of meal was reduced from 53.3% for hexane-extracted meal to 44.3% for the treated seed. The amount of extracted oil remained almost unchanged. The apparent decrease in the yield of meal was brought about by the dissolution of nearly 8% of the low molecular weight polar compounds (such as sugars, phospholipids, phenols, and nonprotein nitrogen compounds) out of the seed and into CH_3OH–NH_3–H_2O, referred to as "gums", thus enhancing the protein content of the resultant meal (Table I).

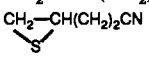
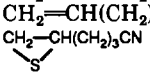
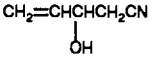
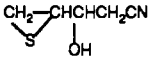
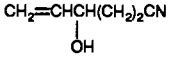
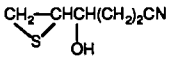
The individual glucosinolates and their concentration in hexane-extracted and in CH_3OH–NH_3–H_2O-treated

Table II. Glucosinolate Content in Hexane-Extracted and CH_3OH-NH_3-H_2O-Treated Altex Meals As Determined by GC and HPLC[a]

glucosinolate	side chain	GC		HPLC	
		hexane-extr	treated	hexane-extr	treated
sinigrin	allyl	0.41 ± 0.03	0.07 ± 0.00	0.51 ± 0.07	0.23 ± 0.05
gluconapin	but-3-enyl	2.43 ± 0.11	0.20 ± 0.02	2.41 ± 0.16	0.22 ± 0.04
glucobrassicanapin	pent-4-enyl	0.23 ± 0.22	0.02 ± 0.00	0.25 ± 0.05	0
progoitrin	2-hydroxybut-3-enyl	4.96 ± 0.22	0.52 ± 0.06	4.61 ± 0.41	0.62 ± 0.11
gluconapoleiferin	2-hydroxypent-4-enyl	0.33 ± 0.03	0.03 ± 0.00	0.40 ± 0.04	0.20 ± 0.04
glucoerucin	4-(methylthio)butyl	0.13 ± 0.01	0.03 ± 0.00	0.10 ± 0.00	0
gluconasturtiin	phenethyl	0.27 ± 0.02	0.06 ± 0.01	0.39 ± 0.06	0
sinalbin	*p*-hydroxybenzyl	0.10 ± 0.01	0		
glucobrassicin	3-indolylmethyl	0.31 ± 0.02	0.03 ± 0.00	3.15 ± 0.22	0.52 ± 0.08
4-hydroxyglucobrassicin	4-hydroxy-3-indolylmethyl	2.40 ± 0.23	0.05 ± 0.00	6.43 ± 0.51	0.91 ± 0.11
glucoalyssin	4-(methylsulfinyl)butyl			0.20 ± 0.02	0
glucoraphanin	5-(methylsulfinyl)pentyl			0.44 ± 0.07	0
total		11.57 ± 0.41	1.01 ± 0.07	18.89 ± 0.72	2.73 ± 0.19

[a] Values are expressed in micromoles per gram of defatted, moisture-free meal ± standard deviation for duplicate injections of three extracted samples. Solvent to seed ratio of 6.7.

Table III. Degradation Products of Aliphatic Glucosinolates in CH_3OH-NH_3-H_2O Treatment[a]

glucosinolate	degradation product	chemical formula
gluconapin	but-3-enyl isothiocyanate	$CH_2{=}CH(CH_2)_2N{=}C{=}S$
	pent-4-enenitrile	$CH_2{=}CH(CH_2)_2CN$
	4,5-epithiopentanenitrile	
glucobrassicanapin	pent-4-enyl isothiocyanate	$CH_2{=}CH(CH_2)_3N{=}C{=}S$
	hex-4-enenitrile	$CH_2{=}CH(CH_2)_3CN$
	5,6-epithiohexanenitrile	
progoitrin	3-hydroxypent-4-enenitrile	
	3-hydroxy-4,5-epithiopentanenitrile	
gluconapoleiferin	4-hydroxy-hex-5-enenitrile	
	4-hydroxy-5,6-epithiohexanenitrile	

[a] Sugar-related degradation products were glucose, thioglucose, its dimer bis(β-D-glucopyranosyl) disulfide, and furfuryl alcohol, in all cases.

Altex meals, determined by GC and/or HPLC, are given in Table II. Their identification was confirmed by comparison with the retention times of known glucosinolates or by comparison with mass and UV spectral data found in the literature (Eagles et al., 1981; Heaney and Fenwick, 1982; Truscott et al., 1982a,b, 1983).

Treatment of crushed seed with CH_3OH-NH_3-H_2O markedly reduced the concentration of glucosinolates in the resultant meal (Table II). However, no apparent preferential trends were observed for the extraction of individual glucosinolates from the meal. A better quantitation of indole-containing glucosinolates was possible using the HPLC method of analysis. Thus, the total content of glucosinolates determined by this method was greater than that determined by GC. Indole glucosinolates are heat sensitive and therefore are underestimated by the GC method of determination. Glucosinolates containing sulfinyl side chains yielded multiple peaks upon GC analysis and thus may be determined only by the HPLC methodology. Other glucosinolates were determined equally well by either method.

To study the fate of the aliphatic glucosinolates in this extraction process, it was necessary to examine whether they were extracted out of the seed as such or were degraded to other products. Due to the complexity of the glucosinolate composition of canola seed and their low concentrations, two pure glucosinolates, namely gluconapin (R = but-3-enyl) and progoitrin (R = 2-hydroxybut-3-enyl), were examined, as the first step. A decrease in their concentration, as shown by a decrease in the absorption intensity at 227 nm, was observed under simulated processing conditions with CH_3OH-NH_3-H_2O. This reduction was 15% for gluconapin and 11% for progoitrin. Thus, gluconapin and progoitrin must have degraded to other products that perhaps did not absorb at 227 nm.

Qualitative studies indicated that sugar-related breakdown products of both model glucosinolates were glucose, furfuryl alcohol, thioglucose, and its dimer (disulfide). The aglycon degradation products were pent-4-enenitrile, but-3-enyl isothiocyanate, and 4,5-epithiopentanenitrile for gluconapin and 3-hydroxypent-4-enenitrile and 3-hydroxy-4,5-epithiopentanenitrile for progoitrin. No oxazolidinethione was produced in this process.

From this information, the fate of the four glucosinolates, namely gluconapin (R = but-3-enyl), progoitrin (R = 2-hydroxybut-3-enyl), glucobrassicanapin (R = pent-4-enyl), and gluconapoleiferin (R = 3-hydroxypent-2-enyl), for which canola seed is generally analyzed, were further studied. Table III summarizes the identity of the aglycon breakdown products of these glucosinolates, and Table IV gives the mass balance of the glucosinolates, their degradation products, and their whereabout after the extraction process.

A close scrutiny of the results indicates that any oxazolidinethione that might have been formed duringthe crushing of seeds is extracted into the polar phase. The major aglycon breakdown products of glucosinolates were nitriles

Table IV. Mass Balance of the Four Specific Canola Glucosinolates As Affected by Solvent Extraction (Micromoles/100 g of Seed, on Dry Basis)[a]

	hexane-extracted		CH_3OH–NH_3–H_2O hexane extracted		
compound	meal	oil	meal	oil	gums
glucosinolates	422.74 ± 1.36	0	41.04 ± 0.29	0	308.42 ± 3.68
desulfoglucosinolates	1.11 ± 0.14	0	0.43 ± 0.07	0	13.56 ± 1.11
isothiocyanates	1.82 ± 0.46	4.32 ± 0.75	0.50 ± 0.14	0.75 ± 0.14	9.92 ± 0.79
oxazolidinethiones	3.68 ± 0.43	5.25 ± 0.82	0	0	8.20 ± 0.75
nitriles	0.54 ± 0.11	0	0.39 ± 0.14	0.89 ± 0.11	52.21 ± 1.82
hydroxy nitriles	0	0	1.14 ± 0.21	0.36 ± 0.07	8.67 ± 1.11
epithio nitriles	0	0	0	0	6.29 ± 0.1

[a] The four glucosinolates were gluconapin, glucobrassicanapin, progoitrin, and gluconapoleiferin.

and hydroxy nitriles, and these were mainly extracted into the polar phase, leaving 1.53 μmol/100 g of seed weight in the treated meal and 1.25 μmol/100 g of seed weight in the extracted oil. The very small amount of 4,5-epithiopentanenitrile that formed during the process (0.08 μmol/100 g of seed weight) was detected in the gums (Table IV).

The amount of intact glucosinolates extracted into the methenolic phase was about 75%. The balance of glucosinolates was either retained in the meal (about 10%) or degraded during the process. Of the decomposed glucosinolates, 93% yielded nitriles, hydroxy nitriles and thioglucose, or its dimer while the remainder produced epithio nitriles, isothiocyanates, and glucose and furfuryl alcohol.

The toxicity of nitriles formed from aliphatic glucosinolates in this process is well described in the literature (Tookey et al., 1980). For example, 3-hydroxypent-4-enenitrile and 3-hydroxy-4,5-epithiopentanenitrile (threo) have LD_{50} values of 170 and 240 mg/kg, respectively. Acute toxicities of these nitriles were much greater than those of oxazolidinethiones (Nishie and Daxenbichler, 1980; Tookey et al., 1980). Poor growth and liver and kidney lessions were observed in rats fed mixed nitriles at 0.1% level for 106 days (Tookey et al., 1980).

The four specific aliphatic glucosinolates studied left a residue of 2.50 mg of nitriles/kg of meal, expressed as 3-hydroxypent-4-enenitrile equivalents. Although this value is lower by nearly 2 orders of magnitude from the LD_{50} values, breakdown of other glucosinolates in canola is expected to increase the total quantity of nitriles in the meals by an estimated factor of 2–3.

The potential application of this process to high-glucosinolate rapeseeds may be limited as larger quantities of nitriles are left in the resultant meals (Shahidi, unpublished results). This may preclude the application of the present process for traditional rapeseeds.

The level of sulfur-containing compounds in the oil due the presence of degradation products of the four aliphatic glucosinolates studied is equivalent to about 0.07 ppm of sulfur. This shows a marked decrease compared to that of 0.9 ppm in the laboratory-prepared hexane-extracted oil.

On the basis of information provided in this paper, further studies on the evaluation of the nutritional quality of the oil and meal obtained from this extraction process is warranted. Efforts toward achieving this goal are currently under way.

CONCLUSIONS

The CH_3OH–NH_3–H_2O hexane extraction of canola produced a meal with enhanced protein content and reduced thyrotoxic potential. The presence of small amounts of nitriles in the meal and in the extracted oil may not lead to nutritional implications for low-glucosinolate rapeseeds of canola variety. Low levels of sulfur in the extracted oil make it a more suitable candidate for hydrogenation than oil produced by the conventional process.

Registry No. NH_3, 7664-41-7; CH_3OH, 67-56-1; sinigrin, 3952-98-5; gluconapin, 19041-09-9; glucobrassicanapin, 19041-10-2; progotrin, 585-95-5; gluconapoleiferin, 19764-03-5; glucoerucin, 21973-56-8; gluconasturtiin, 499-30-9; sinalbin, 20196-67-2; glucobrassicin, 4356-52-9; 4-hydroxyglucobrassicin, 83327-20-2; glucoalyssin, 499-37-6; glucoraphanin, 21414-41-5; but-3-enyl isothiocyanate, 3386-97-8; 4-pentenenitrile, 592-51-8; 4,5-epithiopentanenitrile, 54096-45-6; pent-4-enyl isothiocyanate, 18060-79-2; hex-4-enenitrile, 89464-17-5; 5,6-epithiohexanenitrile, 58130-94-2; 3-hydroxypent-4-enenitrile, 27451-36-1; 3-hydroxy-4,5-epithiopentanenitrile, 30381-35-2; 4-hydroxyhex-5-enenitrile, 76334-68-4; 4-hydroxy-5,6-epithiohexanenitrile, 122567-94-6; glucose, 50-99-7; thioglucose, 22566-82-1; bis(β-D-glucopyranosyl) disulfide, 54495-24-8; furfuryl alcohol, 98-00-0.

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Zonal Distribution of Fatty Acids in Albacore (*Thunnus alalunga*) Triglycerides and Their Changes during Cooking

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The α–β distribution of fatty acids in albacore muscle triglycerides was studied. This analysis was achieved in cooked and uncooked samples in order to assert possible variations during processing. On the other hand, because of their different locations and also different exposures to steam during cooking, three zones of the muscle were considered. Great differences between the α- and β-compositions were observed in the three zones. Fatty acids like 16:0, 18:0, 18:1, 18:3, and 22:1 showed a higher content in the α-position, while 22:6 content was far bigger in β. Due to cooking it was observed that the composition at the β-location suffered more modifications than the α-one. Depending on the zone of the muscle, significant variations were obtained for the main fatty acids.

The first studies on the distribution of fatty acids of triglycerides (TG) of marine fish and invertebrates (Brockerhoff and Hoyle, 1963; Brockerhoff et al., 1963; Dolev and Olcott, 1965) have shown that this lipid class follows the structural pattern typical for most animal fats in which the polyunsaturated fatty acids (PUFA) are preferentially located in the β-position of glycerol. As an explanation of this distribution, it was suggested that the typical structure may be originated in the plankton and then be retained through the food chain. In this point, Brockerhoff et al. (1964) studied the fatty acid distribution in lipids of marine plankton and agreed with this explanation although they pointed out that this may not be the only factor responsible for the typical fatty acid pattern of marine fats.

The characterization of animal fats by fatty acid analysis using the "characteristic" fatty acids ratio has been reported by Litchfield (1972), Carisano and Riva (1976), and Doro (1977). However, three effects make it difficult to recognize the positional distribution of TG: First, dietary fats can alter the composition of animal depot fats (Bishop et al., 1976). Second, the positional distribution patterns are not the same for all kinds of animals. Third, the positional distribution may vary between different body tissues in the same animals.

More recent work in accord with the need for reliable methods to determine the animal species from which processed fish products are made have been published (Takahashi et al., 1978a,b). In them, relations between the main fatty acids of raw and frozen samples which belonging to the different location in triglycerides studied. Later on, Takahashi et al. (1985) developed a mathematical model for the prediction of molecular species in TG by HPLC.

The purpose of the present work was to study the α–β distribution in TG of albacore in order to establish the differences between the raw and cooked material. This species was chosen for its great importance in the Spanish canning industry. Since during cooking the different parts of albacore are not exposed the same way to steam, a zonal study of the muscle (back muscle, ventral

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