Chemical Composition and Toxic Compounds in Rapeseed (*Brassica napus*, L.) Cultivars Grown in Brazil

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The chemical composition, oil characteristics, and glucosinolate, sinapine, and phytic acid contents were studied in low glucosinolate *Brassica napus* varieties, experimentally grown in Brazil. Lipids (43–45%) with an erucic acid content lower than 1% and proteins (18–20%) were the main components. Dietary fiber in meals comprised 23.7–27.5%, when enzymatically determined. Mineral contents were high; dietary fiber and phytic acid can compromise their availability. Sinapine and esters were found at a mean content of 3.4%. Total aliphatic plus indolyl glucosinolates determined by GLC gave values between 26 and 43 μmol/g for air-dried, defatted seed meals, roughly similar to glucosinolate contents determined by enzymatic release and colorimetric measurement of glucose (26–37 μmol/g). Individual glucosinolate analysis showed predominance of progoitrin. The rapeseed varieties introduced in Brazil showed adequate agronomic and chemical characteristics and still offer a promising source of oil and protein for nutritional purposes.

INTRODUCTION

Rapeseed has greatly improved its competitive position in the world because of the efforts to expand and satisfy domestic needs for oil and protein meals.

In addition to its importance as an oil seed, interest in rapeseed as a protein source was first evident when the favorable amino acid composition and a high biological value of the oil-extracted meal was made known (Fenwick, 1982).

The introduction of rapeseed for commercial production in Brazil and its end use for nutritional purposes are largely dependent on the quality control procedures adopted to identify and segregate canola seeds with respect to fatty acid composition, amount and composition of glucosinolates, fiber content, and other antinutritional factors.

It has been recognized that rapeseed shows detrimental physiological effects due to the compounds contained in the seeds, when they are included at high levels in diets, especially for young animals (Fenwick et al., 1983; Fenwick and Curtis, 1980; McDanell et al., 1988). The most important group of harmful substances is glucosinolates and their end products, which partly exhibit toxic effects, characterized by a reduction of feed intake, weight gain, and pathological changes in the thyroid gland, liver, spleen, and other organs. Lesser problems are the presence of phytic acid and sinapine in the meal and the high dietary fiber content (Uppstroem and Svensson, 1980; Fenwick et al., 1984).

Rapeseed oil differs from most other vegetable oils in containing significant amounts of long-chain monoenoic fatty acids. One of these, erucic acid, is undesirable from a nutritional point of view: it causes myocardial lesions and depressed growth in various animals (Slinger, 1977).

With the introduction by Canadian and European breeders of improved varieties of rapeseed containing low levels of glucosinolates and erucic acid (canola varieties), the production of rapeseed as an animal feedstuff has been greatly enhanced (Daun, 1986; Heaney et al., 1988).

Rapeseed with low levels of glucosinolates could be introduced in Brazil as an alternative crop for oil and meal in substitution to wheat and barley production and a way of fully utilizing the capacity of seed-crushing plants in the south of the country during the soybean intercropping.

The recently introduced rapeseed varieties showed adequate agronomic characteristics and good adaptation to Brazilian soils and climates, yielding up to 1.7 ton/ha of grains (Silveira and Da Cunha Gastal, 1985).

The objective of this research was to study the chemical composition of *Brassica napus*, L. seeds, originated from Canadian and German summer cultivars, low in glucosinolates (canola type), with emphasis on protein and oil characteristics and level of antinutritional factors.

In addition, information on erucic acid and glucosinolate contents was needed to assist the grain-crushing plants to establish monitoring programs by quality control and to give them confidence in the utilization of the rapeseed products.

MATERIALS AND METHODS

Rapeseed Cultivars. The original seeds were derived from low-glucosinolate Canadian and European *B. napus*, L. spring cultivars, selected by breeding, and sowed at the Agriculture Experimental Station (EMBRAPA), Passo Fundo, RS. The seeds were experimentally grown during winter (from May until September) without suffering severe frost hazards. One sample was originated from a Canadian (Tower) cultivar and is designated CTC-1; four samples were from German cultivars, Egra (CTC-2) and Erglu (CTC-4, CTC-5, CTC-7).

Preparation of Rapeseed Meals (RSM). Prior to chemical determination, samples from all cultivars were freed from foreign material and ground in a hammer mill to particle sizes of 20 mesh.

The seed meals were continuously extracted with ethyl ether in proportion 1:4 (w/v) during 25 h in a Soxhlet apparatus. The residual meal was air-dried, reground to pass a 60-mesh screen, and re-extracted. The solvent-free rapeseed meals (RSM) were frozen and stored at −20 °C until needed.

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0021-8561/91/1439-1933$02.50/0

Table I. Full Fat Meal Composition (%) of *B. napus* Cultivars[a]

determination	CTC-1	CTC-2	CTC-4	CTC-5	CTC-7
moisture	4.88 ± 0.07	5.04 ± 0.28	5.60 ± 0.30	4.95 ± 0.10	5.16 ± 0.08
ash	3.60 ± 0.01	3.37 ± 0.01	3.62 ± 0.00	3.45 ± 0.01	3.29 ± 0.11
protein (N × 6.25)	19.84 ± 0.40	18.46 ± 1.54	18.87 ± 0.12	18.64 ± 0.43	18.16 ± 0.17
oil (ether extracted)	43.97 ± 0.15	44.67 ± 0.02	43.17 ± 0.14	44.73 ± 0.05	43.77 ± 0.16
ADF	10.70 ± 0.16	13.29 ± 0.40	12.50 ± 0.07	12.16 ± 0.06	14.08 ± 0.22
NDF	12.73 ± 0.16	14.84 ± 0.16	14.22 ± 0.09	13.52 ± 0.12	16.04 ± 0.12
dietary fiber[b]	23.66 ± 0.18	25.49 ± 0.46	24.66 ± 0.42	25.04 ± 0.29	27.46 ± 0.29

[a] Mean ± SD of three determinations. [b] Enzymatic-gravimetric procedure. Mean ± SD of two determinations.

Chemical Determinations. Moisture, protein (N × 6, 25), acid detergent fiber (ADF), and ash content were determined according to Association of Official Analytical Chemists (1980) methods. Neutral detergent fiber (NDF) was determined according to the method of Van Soest and Wine (1967). Dietary fiber was assayed by an gravimetric-enzymatic procedure according to Asp et al. (1983), replacing the Celite 545 used as filter aid by glass wool.

Amino acid analyses were performed according to the method of Spackman et al. (1958) using a modified Honeywell analyzer. RSM samples were hydrolyzed using 6 M HCl in sealed tubes under an atmosphere of N_2, at 110 °C, for 22 h. Cystine and cysteine were determined after oxidation with performic acid to cysteic acid (Moore, 1963). Tryptophan is destroyed by acid hydrolysis and was not determined.

Fatty acid composition was performed by gas chromatography with a Beckman CG 4A instrument equipped with a flame ionization detector and a glass column (3.0 m × 2 mm i.d.) packed with 3% SP-2310 and 7% SP-2300 on Chromosorb W AW 100/120 mesh at 200 °C; injector and detector temperatures were 250 °C. The carrier gas was helium, at a flow rate of 30 mL/min. Methyl esters were prepared from oils, according to American Oil Chemists' Society (1971) methods. Peaks were identified by comparison with authentic standards, quantitated by peak area integration, and expressed as weight percent of total methyl esters; the relative weight percent of each fatty acid was determined from integrated peak areas.

Total Glucosinolate Analysis. The RSM were analyzed for intact glucosinolates by the enzymatic procedure of Van Etten et al. (1974). Glucosinolates were hydrolyzed by incubation for 1 h, at 35 °C, with thioglucosidase (myrosinase) prepared from white mustard seed (*Sinapis alba*, L.) according to the method of Appelqvist and Josefsson (1967). The suspension was allowed to stand for 1 h, and the clean supernatant was taken for glucose determination. The amount of glucose was estimated according to the method of Bergmeyer and Bernt (1974) using 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) as chromogen. The total glucosinolate contents were expressed in micromoles per gram of RSM and calculated from a glucose calibration curve, assuming that 1 mol of glucose corresponded to 1 mol of progoitrin, the most predominant glucosinolate found.

Gas–Liquid Chromatography of Glucosinolates. Individual trimethylsilylated glucosinolates were estimated by temperature-programmed gas–liquid chromatography after extraction, purification by ion-exchange chromatography, and enzymatic removal of sulfate groups present in glucosinolates. The method is described by the Canadian Grain Commission Grain Research Laboratory (Daun and McGregor, 1981) and is a modification of the Heaney and Fenwick (1980) procedure.

For calculating the mole composition of glucosinolates (aliphatic and indolyl) present in the silylated samples, we assumed the extent of derivatization would be the same for each glucosinolate and their mole compositions were proportional to peak areas divided by the number of carbons present in the trimethylsilyl derivative. Benzyl glucosinolate was used as internal standard, and a correction for the relative response of benzyl to aliphatic glucosinolate was made. Results are expressed in micromoles of glucosinolate per gram of RSM (moisture content was 8.2–10.7% of meal). Two indolyl compounds were resolved in the same chromatogram and identified as 3-indolylmethyl and *N*-methoxy-3-indolylmethyl. Their contents were expressed as the sum of both.

Total Sinapic Acid Esters. RSM were analyzed for sinapine plus sinapic acid esters according to the methanol extraction method of Mueller et al. (1978). The filtered and combined methanol extracts were diluted (1:50) with acidified methanol, and optical density was determined at 326 nm. The concentrations of sinapine and sinapic acid esters were assessed by referencing optical densities obtained for unknowns to a standard curve of sinapine bissulfate over a 0–20-μg range, according to the Clandinin (1961) procedure. The sinapine content includes all sinapic acid esters plus free sinapic acid.

Phytic Acid. Phytic acid was extracted and determined according to the supernatant difference method of Thompson and Erdman (1982), with minor modifications. A 1-g sample of RSM was extracted with 25 mL of 3% TCA containing 10% Na_2SO_4, by mechanical shaking during 30 min. The suspension was centrifuged at 12100*g* for 15 min in a Sorvall RC2-B centrifuge. The supernatant was decanted and the pellet re-extracted for 30 min more. After centrifugation, the supernatants were collected and the phytic acid was precipitated with $FeCl_3$ solution. The amount of phytic acid was obtained by difference of the phosphorus value (Fiske and Subbarow, 1925) between the initial supernatant and after precipitation with $FeCl_3$. The conversion factor 3.55 for phosphorus to phytic acid was used.

Mineral analyses were as those described by Perkin-Elmer Corp. (1976). A Perkin-Elmer Model 373 instrument was used. Ashing procedures of the Instituto Adolfo Lutz (1985) have been adopted.

Statistical Analysis. Analysis of variance and Tukey's significant difference test were used to assess statistical significance (Neter et al., 1985).

RESULTS AND DISCUSSION

Some of the chemical characteristics of the analyzed rapeseed cultivars are shown in Table I. Major components are lipids (43–45%), proteins (18–20%), and a dietary fiber fraction (24–27%).

The dietary fiber determined according to the enzymatic-gravimetric method showed mean values 78% higher than those determined according to the NDF method and 102% higher than those according to the ADF method. Soluble fiber components were removed by EDTA of the neutral detergent solution and were responsible for the low NDF values. Consequently, the NDF and ADF failed as predictors of the apparent metabolizable energy values of various feedstuffs, including rapeseed, because it is rich in soluble fiber, mainly pectic substances (Carré and LeClercq, 1985; Carré and Brillouet, 1986; Carré et al., 1984).

Therefore, we recommend the enzymatic-gravimetric procedure to determine the fiber fraction that permits also the estimation of the available carbohydrates in rapeseed with more accuracy.

The composition of the rapeseed meals obtained from solvent extraction is given in Table II. Protein content varied between 32.4 and 34.5%, and the meals contain only residual lipids (<1%). The three (CTC-1, CTC-4, and CTC-5) rapeseed meals analyzed correspond to the minimum quality criteria specifications of the Canola Council of Canada (Campbell, 1984) for canola meals, which established a minimum protein content of 34.0% by mass.

Table II. Rapeseed Meal and Oil Characteristics of Brazilian Cultivars[a]

determination	CTC-1	CTC-2	CTC-4	CTC-5	CTC-7
		Meal Composition			
moisture, %	10.7	9.3	8.2	8.5	8.7
residual lipids, %	0.3	0.3	0.2	0.3	0.3
protein,[b] %	34.5	33.2	33.7	33.8	32.4
glucosinolates,[c] μmol/g (enzymatic method)	31.76 ± 0.00	26.36 ± 0.73	36.92 ± 0.73	25.85 ± 0.73	33.82 ± 0.00
sinapine plus sinapic acid esters, %	2.7	3.3	4.6	3.1	3.3
phytic acid, %	3.2	3.2	3.4	3.4	2.9
minerals					
calcium, %	0.50	0.53	0.57	0.52	0.59
phosphorus, %	1.39	1.36	1.52	1.55	1.27
iron, %	0.12	0.09	0.05	0.08	0.10
sodium, %	0	0	0	0	0
magnesium, %	0.86	0.84	0.78	0.84	0.85
potassium, %	1.41	1.08	1.20	1.19	1.03
zinc, ppm	70	57	52	51	62
copper, ppm	266	262	21	33	292
manganese, ppm	70	69	64	67	77
		Oil Composition			
major fatty acid					
palmitic (16:0), %	0.4	0.4	0.4	0.4	0.4
oleic (18:1), %	58.8	60.6	60.6	57.2	60.1
linoleic (18:2), %	21.9	18.7	20.3	19.0	20.0
linolenic (18:3), %	8.6	8.9	8.6	10.3	8.8
eicosenoic (20:1), %	1.5	2.3	1.4	3.6	1.6
erucic (22:1), %	0.1	0.9	0.1	0.9	0.3

[a] Mean of duplicate determinations. [b] Calculated from seed protein content. [c] Mean ± SD of two determinations.

Table III. Amount of Individual Aliphatic and Indolyl Glucosinolates in Rapeseed Meal by Gas–Liquid Chromatography[a]

	μmol/g				
glucosinolate	CTC-1	CTC-2	CTC-4	CTC-5	CTC-7
3-butenyl gluconapin	7.87[b] ± 0.31	4.89[a] ± 0.40	8.89[c] ± 0.20	5.25[a] ± 0.02	8.19[b] ± 0.03
4-pentenyl glucobrassicanapin	0.90	1.25	1.67	0.95	1.58
2-hydroxy-3-butenyl progroitrin	16.34[c] ± 0.01	11.26[a] ± 0.18	21.91[e] ± 0.45	12.41[b] ± 0.17	20.65[d] ± 0.26
2-hydroxy-4-pentenyl napoleiferin	0.66	0.84	1.19	1.17	0.83
total aliphatic glucosinolates	25.77[c] ± 0.24	18.24[a] ± 0.40	33.66[e] ± 0.64	19.78[b] ± 0.12	31.20[d] ± 0.14
indolyl glucosinolates	7.31[a] ± 1.96	7.59[a] ± 0.22	10.04[a] ± 1.73	9.04[a] ± 1.41	6.07[a] ± 0.16
total aliphatic and indolyl glucosinolates	33.08[bc] ± 1.72	26.07[a] ± 0.96	43.59[d] ± 0.94	28.39[ab] ± 1.90	37.27[c] ± 0.99

[a] Mean ± SD of two determinations. Means not sharing a common superscript letter along a horizontal line differ significantly ($P < 0.05$).

Rapeseeds are characterized by the presence of several antinutritional factors that have adverse effects on animal performance. These include mainly glucosinolates or thioglucosides and are one of the limiting factors in the use of rapeseed meal in animal diets.

Total glucosinolate contents (aliphatic and indolyl) (Table II) determined according to the enzymatic method were between 26 and 37 μmol/g and compared approximately with the sum of individual aliphatic plus indolyl glucosinolates estimated by gas–liquid chromatography (26–44 μmol/g) (Table III). The two methods, among others, are currently in use for determining the glucosinolate content of rapeseed meals.

On the basis of nutritional studies, the Canola Council of Canada (Campbell, 1984) established the maximum level of 30 μmol/g of oil-free meal for the four main aliphatic glucosinolates (3-butenyl, 4-pentenyl, 2-hydroxy-3-butenyl, and 2-hydroxy-4-pentenyl).

The data presented in Table III indicate the values obtained by gas chromatographic analysis for individual glucosinolates. The relative amounts of 3-butenyl, 4-pentenyl, and 2-hydroxy-4-pentenyl constituted only a small percentage of the total glucosinolate content, while the 2-hydroxy-3-butenyl constituted over 60%. The amounts of individual glucosinolates were relatively low but variable in the different cultivars. The CTC-2, CTC-5, and CTC-1 cultivars showed the lowest aliphatic glucosinolate contents, respectively, 18.24, 19.78, and 25.77 μmol/g of RSM. We found that three of the analyzed rapeseed meals could be considered of Canola-type quality. The other two produced meals just above Canola specifications. The methodology (gas–liquid chromatography) used to measure individual aliphatic and indolyl glucosinolates cannot resolve adequately the indolyls, and contradictory reports exist regarding its structure (Slominski and Campbell, 1987). For this reason the indolyls detected (Table III) are summed and reported together to assess the differences of amounts between aliphatic and nonaliphatic glucosinolates. The indolyls were shown to be present in all of the seed samples and averaged 8.0 μmol/g of meals. Further studies on the chemical identity and nutritional aspects are necessary. Indolyls plus their hydrolysis products have been implicated with anticarcinogenic activities due to activation of some metabolizing enzyme systems in various body tissues of experimental animals. A possible hazard from indolyls in diets is their reaction with nitrites to form carcinogenic *N*-nitroso compounds (McDanell et al., 1988).

Sinapic acid, another antinutritional component present in rapeseeds, is derived from sinapine, which has a bitter taste and may influence palatability, and is also involved in reactions with proteins, reducing their nutritive value. This problem is particularly enhanced when large amounts of RSM are included in poultry rations (Fenwick and Curtis, 1980). The spectroscopic properties of methanol extracts of defatted RSM were used for quantification of the sinapine content. Sinapine and sinapine esters (Table II) were present in variable amounts (2.7–4.6%) slightly

Table IV. Amino Acid Content of Rapeseed Meals[a]

amino acid	g/16 g of N				
	CTC-1	CTC-2	CTC-4	CTC-5	CTC-7
lysine	5.78	5.84	6.01	5.98	6.35
histidine	3.00	2.97	3.08	3.02	3.26
arginine	6.20	6.04	6.60	6.44	6.74
aspartic acid	8.78	8.20	8.39	7.97	8.96
threonine	5.94	5.60	5.69	5.67	5.89
serine	5.51	5.40	5.61	5.29	5.89
glutamic acid	17.41	18.03	19.38	18.20	18.11
proline	6.93	8.01	5.84	7.44	6.20
glycine	5.72	5.46	5.64	5.39	6.04
alanine	5.44	5.07	5.18	4.90	5.64
half-cystine	1.39	1.52	1.59	1.73	2.07
valine	4.89	4.75	4.52	5.09	4.01
methionine	2.32	2.66	2.51	2.45	2.07
isoleucine	4.15	3.86	3.46	4.04	3.28
leucine	7.83	7.72	7.90	7.75	7.64
tyrosine	3.07	3.12	3.05	3.00	2.66
phenylalanine	4.32	4.31	4.13	4.21	3.77

[a] Mean of duplicate samples hydrolyzed for 22 h.

higher than those reported by other authors (Blair and Reichert, 1984), probably due to the nonspecific analytical method we used, that include other phenolic compounds.

The mineral analysis of the RSM, also shown in Table II, confirms that rapeseed meals are richer sources of minerals than soybean meals as reported by Fenwick (1982). However, the presence of a high dietary fiber level and the presence of about 3% phytic acid which provides approximately 65% of the total phosphorus in the seeds can compromise significantly the availability of minerals. Zinc bioavailability is the most affected (Nwokolo and Bragg, 1980). The presence of a large variation in copper content, between 21 and 292 ppm, confirmed data presented by Hougen and Stefansson (1982), who reported a copper content from 5 to 700 ppm.

The composition of the major fatty acids of the rapeseed oil is summarized in Table II. The fatty acid composition of the analyzed rapeseed oils showed, as expected for "canolas", a reduced content of the undesirable erucic acid, in agreement with the recommendations of the Rapeseed Association of Canada (<1%). The presence of glycerides derived from the high linolenic acid content (>8%) which are readily oxidized can produce unacceptable off-flavors.

Despite the adverse features of the meal, there is a good balance of essential amino acids. The amino acid composition of the RSM proteins is shown in Table IV. Lysine content is 6% on average and in agreement with previously published data (Hougen and Stefansson, 1982). The combined sulfur amino acids (methionine and cysteine) occur at levels of 3.7–4.2% in the analyzed samples. Lysine can become limited when meals for animal nutrition are prepared under severe heating processing.

From a biochemical point of view, the meals obtained from the CTC-2 and CTC-5 cultivars showed the lowest glucosinolate contents, which permitted their classification into canola grade standards, while the CTC-1 and CTC-4 extracted oils were lowest in erucic acid.

ACKNOWLEDGMENT

The present study was supported by a research grant (Project. 03683031/4) from EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária), Brazil. We are grateful to Dr. L. J. Greene for conducting the amino acid analysis and to Dr. O. Szenebaum for some of the mineral analysis. The gas chromatographic glucosinolate and fatty acid measurements were conducted at Agriculture Canada Research Station, and their support is greatly appreciated.

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Received for review November 20, 1990. Revised manuscript received June 29, 1991. Accepted August 2, 1991.

Registry No. Sinapine, 18696-26-9; sinapic acid, 530-59-6; phytic acid, 83-86-3; calcium, 7440-70-2; phosphorus, 7723-14-0; iron, 7439-89-6; sodium, 7440-23-5; magnesium, 7439-95-4; potassium, 7440-09-7; zinc, 7440-66-6; copper, 7440-50-8; manganese, 7439-96-5; gluconapin, 19041-09-9; glucobrassicanapin, 19041-10-2; progroitrin, 585-95-5; napoleiferin, 19764-03-5.