Microwave Treatment of Dehulled Rapeseed to Inactivate Myrosinase and Its Effect on Oil and Meal Quality

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ABSTRACT

Rapeseed was dehulled using a Palyi pneumatic attrition system which produced 62-66% clean dehulled seed. Dehulled rapeseed was preconditioned to 7, 10 and 13% moisture levels, exposed to microwave irradiation for periods of up to 2.5 min and analyzed for residual thioglucoside glucohydrolase (myrosinase) activity. The 7% moisture samples heated slowly and required at least a 2.5 min treatment whereas 10 and 13% moisture samples heated more rapidly and required microwave exposures of 1.5 min or less for complete inactivation of the enzyme. The sulfur content of oils obtained from adequately microwave-treated samples (1.5 min for 10 and 13% and 2.5 min for 7% moisture samples) was equal to or lower than commercially processed crude rapeseed oils. The shorter microwave treatment of dehulled rapeseed produced considerably lighter oils and did not adversely affect the color of the meal. It also destroyed some of the rapeseed glucosinolates and improved the meal palatability. However, goitrogenic properties of microwave-treated rapeseed meal evaluated by mice feeding experiments did not appear different from untreated rapeseed meal.

INTRODUCTION

The oilseed referred to as rapeseed on the world market is derived from *Brassica napus*, *B. campestris* and *B. juncea* species of the Cruciferae family (1). From the processor's point of view, the kernel can be considered to consist of an outer hull and the inner meat. The hull is fibrous and varies in color from yellow to brown or black depending on its content of polyphenols (2,3). The dark color of hulls is objectionable since it adds to the color of the crude oil and the defatted meal. Hulls also contribute significantly to the crude fiber content of the meal. In attempting to overcome the problems associated with hulls in rapeseed processing, plant breeders have developed yellow-hulled varieties which also have lower contents of crude fiber and polyphenols (3). In addition, various methods of dehulling rapeseed have been developed (4,5).

The meat portion of the seed contains several undesirable and antinutritional factors such as glucosinolates (6), phytates (7), polyphenolic compounds (8), odoriferous and bitter principles (9,10), indigestible carbohydrates (11) and other unidentified heat labile constituents (12-14). The glucosinolates in rapeseed are the source of goitrogens (6,15,16) and sulfur contamination of the oil (17). Glucosinolates are insoluble in oil but their hydrolytic products resulting from the action of an endogenous enzyme, thioglucoside glycohydrolase (EC 3.2.3.1) or myrosinase, and heat (18) are oil soluble. Rapeseed processors therefore inactivate this enzyme using a dry heat treatment (19-21) in which crushed rapeseed is heated to 80-90 C as rapidly as possible to facilitate the extraction of oil (22). However, commercially processed crude and refined rapeseed oils contain 10-57 and 3-5 ppm sulfur (18), respectively, suggesting some hydrolysis of glucosinolates is inevitable in this process. Sulfur in rapeseed oil is objectionable as it poisons the hydrogenation catalyst (23,24) and produces unpleasant odors during heating and hence is unfavorable to the economics of rapeseed oil production.

In attempting to minimize the problem of sulfur contamination of rapeseed oils, some studies in the past were directed toward rapid heat inactivation of the enzyme in the intact seed (19). Microwave treatment of rapeseed seeds was one of the means considered for this purpose since it results in extremely rapid heating of the material. The studies of Eapen et al. (19) with whole rapeseed and Armstrong (25) with dehulled rapeseed showed the effectiveness of a short microwave treatment in inactivating the enzyme, but Eapen et al. also reported its adverse effect on the quality of the oil and the meal. The effect of this treatment on glucosinolate content of rapeseed was not reported, although it is known that calcination results in substantial destruction of goitrogens (13,26). The thermal decomposition of glucosinolates in dry, aqueous and model systems has also been reported (27-29), as has been the influence of microwave heating on antinutritional factors in soya proteins (30).

We have investigated microwave processing of dehulled rapeseed and report here conditions for microwave treatment to inactivate the enzyme thioglucoside glucohydrolase and its effect on the sulfur content and color of crude oil, as well as the glucosinolate content and color of the defatted meal. Results of biological evaluation of microwave-treated rapeseed meal relative to the untreated rapeseed meal also are included.

MATERIALS AND METHODS

Canadian-grown rapeseed seeds of *Brassica napus* cultivar Tower, *B. campestris* cultivar Candle and *B. Juncea* (oriental mustard) were obtained from the Saskatoon Research Station of Agriculture Canada. Commercial samples of crude oil and meal from Tower and Candle rapeseed were obtained from the Food Research Institute, Ottawa, and Canbra Foods Ltd., Alberta, respectively. A commercial sample of *B. juncea* (mustard) oil was purchased locally.

Dehulling of Seeds

The seeds were dehulled in 22.7-kg batches using the Palyi pneumatic small-seed dehulling unit (31) which yielded 5 fractions: clean dehulled meat, hulls, hulls and meat, fines and undehulled seeds and splits in yields of 62-66, 2-2.5, 22-25, 5-6 and 3-6%, respectively. Material losses of up to 0.5% were encountered. The clean dehulled meat fraction, hereafter referred to as dehulled rapeseed, was stored at 0-4 C until required.

Proximate Analysis

The proximate analysis of rapeseed samples was performed using AOAC standard procedures (32).

Microwave Treatment

Duplicate lots of dehulled material in 300-g quantities were adjusted to moisture contents of 7, 10 and 13% (± 0.15%), equilibrated at 0-4 C for 24 hr and exposed to 2450-MHz microwave irradiation in 40-g quantities in 400-ml glass beakers in a 1.25-KW Westinghouse microwave oven for periods of 1, 1.5, 2 and 2.5 min (29). The moist microwave-

treated and untreated samples were air dried at room temperature. The samples were ground in a Wiley mill to 20 mesh and defatted in a Soxhlet apparatus using *n*-hexane for 12 hr. The defatted meal was desolventized using air current at room temperature and pulverized to 60 mesh. Solvent from the hexane extracts was removed using a rotary evaporator and the residual oil was clarified by centrifugation prior to analysis.

Thioglucoside Glucohydrolase Activity in Meal

A vial containing 2 ml of citrate-phosphate buffer (pH 7.0, 0.18 M), 2 ml of methylene chloride and 0.25 g of the sample meal was shaken for 4 hr at 22 C. The vial was then centrifuged to obtain a clear layer of methylene chloride. The aglucons content of this layer was analyzed by HPLC (33). The amount of the aglucons liberated per unit weight of the meal was taken as an index of the thioglucoside glucohydrolase activity in the sample analyzed.

Sulfur Content of Oils

The sulfur content of oil samples was determined by the Raney nickel method (34) using apparatus and procedural modifications as recommended elsewhere (17,35).

Color of Oils

Tower, Candle and *B. juncea* oil samples were diluted with carbon tetrachloride by factors of 20, 10 and 10, respectively. The diluted oil samples were scanned over the visible range using a Model 35 Beckman spectrophotometer with a 10-mm light path. Using the same cuvette, the samples were also measured for their yellow, red and blue color units with the Lovibond Tintometer Type D (Tintometer Ltd., Salisbury, England).

TABLE I

Composition of Basal Diet

Ingredient	%
Sucrose	10.0
Dextrose	10.0
Corn oil	10.0
Cellulose	5.0
Salt mix[a]	3.5
Vitamin mix[b]	2.2
Starch	59.3
Total	100.0

[a]Williams-Brigs modified. Purchased from Grand Island Biological Company, Grand Island, NY.

[b]See Srivastava et al. (38) for composition.

Color of Meals

The color of meal samples was measured by reflectance using a Hunterlab D25D L Color and Color-difference Meter (Hunter Associates Laboratory Inc., Fairfax, VA).

Glucosinolate Content of Meals

Glucosinolate content was determined by HPLC (33) using acetonitrile and water in 60:40 proportion (v/v) as the solvent. Peak height was used as a measure of aglucon concentration.

Nutritional Studies

A commercial sample of dehulled *B. napus* was soaked in tap water (1:10) for 12 hr at room temperature. The wet, drained sample was spread on paper plates in uniform layer of ca. 1 cm thickness and exposed to 2450-MHz microwave irradiation in a 125-KW, Litton microwave oven, Model 550, for 4.5 min (25). The untreated and the microwave-treated samples were freeze dried, ground and defatted using *n*-hexane. The glucosinolate content of these meal samples was determined spectrophotometrically (36).

White Swiss male mice were obtained at 21 days of age from Connaught Laboratories Ltd., Toronto, and fed for 10 days on commercial rat chow before randomly assigning them in groups of 8 to experimental diets. The average weight of mice at the start of the experimental period was ca. 20 g. The feeding trial procedure was similar to that used by Lo and Hill (37) with some modification in the mineral and vitamin mixtures used in the basal diet (Table I). Each of the 2 rapeseed preparations and the methionine supplemented casein (+ 3.2% DL-methionine), used as a standard, contributed 8% protein (N x 6.25) to the respective experimental diets and replaced a portion of the corn starch to retain the total of the dietary ingredients at 100%. The experimental diets were fed for 15 days.

RESULTS AND DISCUSSION

Proximate composition and glucosinolate content of whole and dehulled rapeseed seeds are presented in Table II. These figures indicate that the dehulled material contained appreciably more lipid and glucosinolates and less crude fiber and nitrogen-free extract as compared to the parent seeds. The protein and the mineral constituents stayed relatively constant.

Loss of weight (apparently moisture) from the rapeseed samples during microwave treatment was taken as an index of the rate of microwave heating. Experimental results for this loss from 7, 10 and 13% moisture samples are presented in Figure 1. There were no statistically significant differences ($P \leq 0.05$) among the 3 *Brassica* species studied and each point with a vertical bar in this plot represents

TABLE II

Composition of Whole and Dehulled Tower, Candle and *B. juncea*[a]

Sample	Proximate components (% dry basis)					
	Crude fat	Protein (N x 5.53)	Crude fiber	Mineral matter	Nitrogen-free extract (by difference)	Glucosinolates (mg aglucons/g of dry, defatted meal)
Tower	41.56	22.71	5.03	4.31	26.39	2.32
Dehulled Tower	49.18	23.38	2.07	4.33	21.04	2.96
Candle	44.31	18.71	5.39	3.92	27.67	1.52
Dehulled Candle	50.29	18.94	3.05	3.75	23.97	2.02
B. juncea	33.86	27.33	5.05	4.48	29.28	22.60
Dehulled *B. juncea*	43.68	26.63	2.93	4.13	22.63	28.37

[a]Average of duplicates.

the mean and standard deviation of 6 samples. The results indicated a comparatively lower absorption of microwave energy by the 7% moisture sample and an increase in rate of microwave heating with increasing initial moisture levels in the samples. These results are in good agreement with our previous studies with model soya systems (29) and demonstrate the importance of preconditioning for effective absorption of microwave energy by the samples during such treatments.

Microwave treatment of rapeseed samples preconditioned to 10% moisture level for a period of 1.5 min destroyed the thioglucoside glucohydrolase activity completely (Table III). Preconditioning of samples to 13% moisture levels lowered the exposure requirement to 1.0 min in *B. juncea*. Samples with an initial moisture level of 7% required longer exposure periods for enzyme inactivation. At this moisture level, a 2.5 min microwave exposure period was effective in destroying the enzyme completely only in Tower and *B. juncea* samples. These results confirm the comparatively lower absorption of microwave energy by the 7% moisture samples. The results also suggest that the minimum microwave treatment required to inactivate the enzyme may be species-dependent.

The oil samples were analyzed for their sulfur and color contents. The sulfur content of the crude oil samples was affected not only by the rapeseed type, period of exposure to microwave energy, the adjusted level of initial moisture in the samples and their 2- and 3-factor interactions, but also by the preconditioning step in preparation of the samples (Figure 2). In the control samples, the sulfur content of the oils increased with increasing levels of moisture in all 3 *Brassica* species, indicating the moisture dependency of thioglucoside glucohydrolase activity in dehulled material which is bruised during the dehulling process (31). On the average, the sulfur content of oil samples was minimal for Candle, maximal for *B. juncea* and intermediate for Tower, which correlates well with their glucosinolate levels (Table II). The sulfur content of oils increased with increasing periods of microwave exposure. This increase was greater with increasing initial moisture contents of the samples in all 3 *Brassica* species. From these results it was concluded that thioglucoside glucohydrolase activity during initial exposure periods and thermal decomposition of glucosinolates contributed to the sulfur content of rapeseed oils. The sulfur content of oils from adequately microwave-treated, dehulled samples (1.5 min for 10 and 13%, and 2.5 min for 7% moisture samples) was lower than the corresponding commerical crude oil samples of Tower, Candle and mustard which were found to contain 12.3, 33.5 and 395 ppm of sulfur, respectively, although the history of these Tower, Candle and mustard samples is not known.

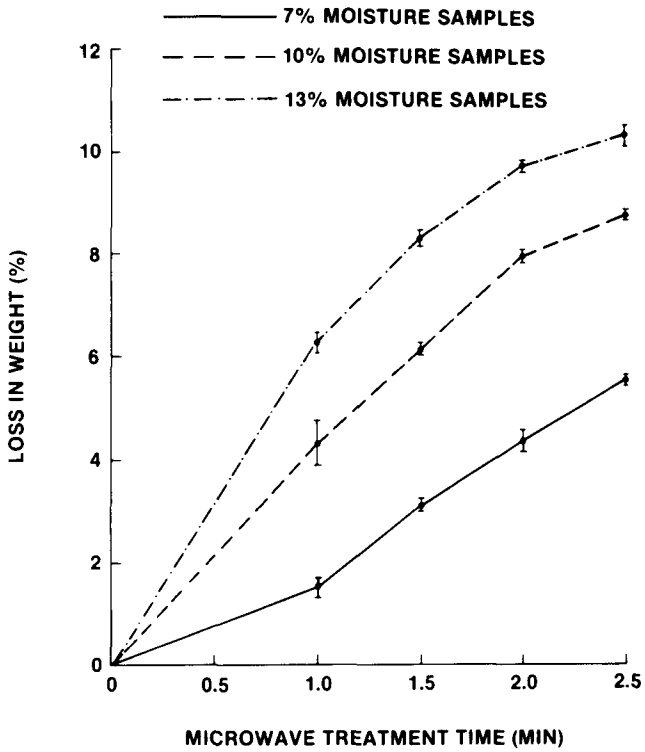

FIG. 1. Weight loss from Tower, Candle and *B. juncea* samples as a function of moisture content and duration of exposure to microwave energy.

All oil samples were scanned from 360 to 760 nm and the scans were examined for any gross change in their absorption pattern as a consequence of microwave treatments. The scans of the control and the microwave-treated oil samples were similar with the exception of some minor differences in the intensities of 4 major peaks observed in the spectra of all samples at λ maxs. of 670, 482.5, 455

TABLE III

Thioglucoside Glucohydrolase Activity in Microwave-treated Tower, Candle and *B. juncea*[a]

Adjusted initial moisture level	Microwave treatment time (min)	Residual enzyme activity[b]		
		Tower	Candle	*B. juncea*
7	1.0	100	100	100
	1.5	95	94	20
	2.0	46	11	1
	2.5	0	1	0
10	1.0	96	78	23
	1.5	0	0	0
	2.0	0	0	0
	2.5	0	0	0
13	1.0	21	5	0
	1.5	0	0	0
	2.0	0	0	0
	2.5	0	0	0

[a]Average of 2 samples per treatment combination.

[b]Values relative to control sample (no microwave treatment) which was assigned an index score of 100.

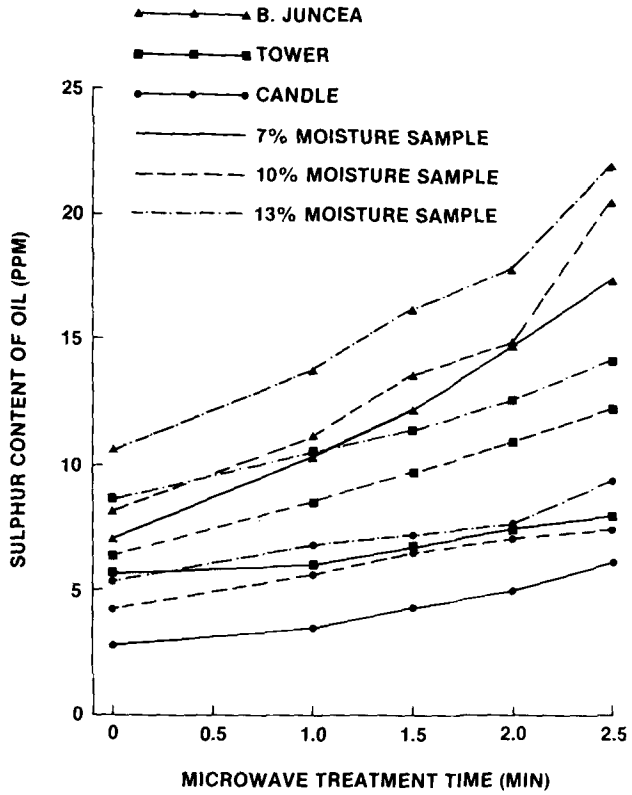

FIG. 2. Sulfur content of crude Tower, Candle and *B. juncea* oils as a function of moisture content and duration of exposure to microwave energy.

and 427 nm. These differences were reflected in visual color content of diluted samples as assessed by the Lovibond Tintometer. The absence of blue color units in oils from microwave-treated samples indicated that these treatments did not result in any darkening of the oil color. Analysis of the commercially important red color content of microwave-treated oil samples showed that only Tower species was influenced. Microwave processing increased the red color content from 0.3 units for the unheated rapeseed to 0.4 units for all processed samples. The experimental data on yellow color content of oil samples from each species were analyzed statistically using *n*-way classified analysis of variance and Dunnett's and Tukey's ω-procedures for comparisons of treatment means. There were no significant differences ($P \leq 0.05$) in yellow color content of oils from all adequately microwave-treated samples and their respective controls. The differences among 7, 10 and 13% moisture samples within each species were also insignificant.

The experimental oil samples from dehulled Tower and Candle rapeseed were lighter and lower in red (R) and yellow (Y) color content (0.4R + 6.8Y for Tower oil and 0.2R + 10.0Y for Candle oil) than the commercial samples (0.5R + 20.3Y + 0.1 blue color units for Tower oil and 0.8R + 20.2Y + 0.1 blue color units for Candle oil) which were extracted from undehulled material. From our results and those reported by Eapen et al. (19) it appears that hulls contribute much more to color than the short heat treatment necessary to inactivate the enzyme thioglucoside glucohydrolase and that darker oil color results from heat treatment and crushing of whole seeds.

Meal samples were analyzed for their color; their color values on Hunter L, a and b scales measuring their lightness, redness or greenness and yellowness, respectively, are presented in Table IV. The lightness values (L) of meals from adequately microwave-treated samples of each of the 3 *Brassica* species did not differ significantly from their respective controls. Only the 2.5 min microwave-treated Tower meal from 7% moisture samples was significantly less green (a = -1.1) than its control (a = -1.9); all other a values showed no significant change from the treatment. The b values for microwave-treated samples from the 3 species were higher than their respective untreated samples. However, the b values of meals from adequately microwave-treated samples of Tower and *B. juncea,* but not Candle, were significantly higher than their untreated samples. These results suggest that the influences of initial moisture content and microwave treatment on the color of meal are species-dependent. The short microwave treatments required to inactivate the enzyme myrosinase did not have any significant adverse effect on the color of meal from the 3 species studied.

The commercial meal samples of Tower and Candle rapeseed with L, a and b values of 43.34, 6.92 and 15.80, and 43.47, 5.97 and 16.26, respectively, were dark and reddish brown in color resulting from the presence of hulls and hence were unsuitable for any color comparison with the experimental meal samples from dehulled rapeseed.

Preconditioning and microwave heating of rapeseed

TABLE IV

Color Content of Tower, Candle and *B. juncea* Meals As a Function of Moisture Content and Duration of Exposure to Microwave Energy[a]

Sample	Hunter color scale	Moisture levels (%)			Microwave treatment time (min)					Overall mean
		7	10	13	0.0	1.0	1.5	2.0	2.5	
Tower	L[b]	86.4[c]	87.1[d]	87.3[d]	86.7[e,f]	87.4[f,g]	86.4[e]	87.8[g]	86.7[e,f]	87.0
	a	-1.5[d]	-2.0[c,d]	-2.5[c]	-2.1[e,f]	-2.4[e]	-1.9[e,f]	-2.2[e,f]	-1.5[f]	-2.0
	b	20.2[c]	20.5[c,d]	20.7[d]	19.9[e]	20.7[f]	20.8[f]	20.2[e,f]	20.7[f]	20.5
Candle	L	87.8[c]	88.3[c]	88.2[c]	88.9[f]	86.2[e]	88.8[f]	88.3[f]	88.3[f]	88.1
	a	-1.9[d]	-2.1[c,d]	-2.3[c]	-2.2[e,f]	-1.7[f]	-2.3[e,f]	-2.1[e,f]	-2.1[e,f]	-2.1
	b	18.5[d]	17.8[c]	17.8[c]	17.3[e]	19.0[f]	17.9[e]	17.9[e]	18.0[e]	18.0
B. juncea	L	90.1[c]	90.0[c]	90.2[c]	90.9[f]	89.5[e]	90.8[f]	89.6[e]	89.7[e,f]	90.1
	a	-2.6[c]	-2.6[c]	-2.8[c]	-2.9[e,f]	-2.4[f,g]	-3.1[e]	-2.3[g]	-2.5[f,g]	-2.6
	b	19.0[c]	18.8[c]	18.9[c]	17.9[e]	19.5[f]	19.1[f]	19.1[f]	18.9[f]	18.9

[a]Mean values of 5 or 3 determinations.

[b]See Results and Discussion for an explanation of terms.

[c,d]and [e,f,g]Rows within moisture levels or microwave treatment times bearing similar superscripts do not differ significantly ($P \leq 0.05$) using Tukey's ω-procedure.

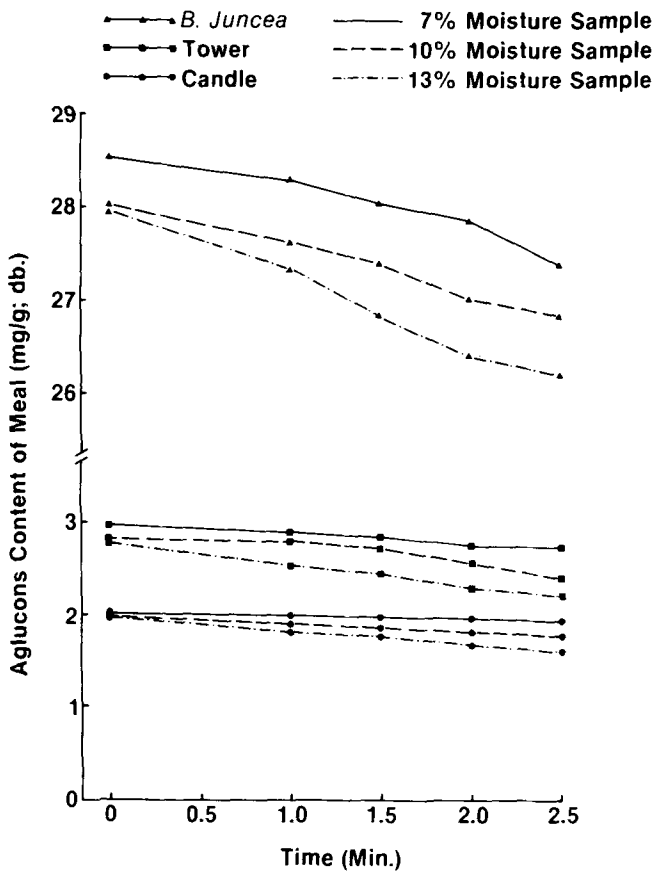

FIG. 3. Decomposition of glucosinolates in Tower, Candle and *B. juncea* as a function of moisture content and duration of exposure to microwave energy.

resulted in some decomposition of glucosinolates (Figure 3). This decomposition increased with increasing moisture content of samples in all 3 *Brassica* species. The decomposition also increased with increasing periods of exposure to microwave energy. The maximal decomposition occurred in 13% moisture samples after 2.5 min exposure to microwave energy and averaged 8.2% for *B. juncea,* 25.4% for Tower and 24.7% for Candle rapeseeds. This decomposition of glucosinolates coincided with an increase in sulfur content of oil samples, suggesting that some decomposition products of glucosinolates enter the oil and lower its processing quality.

The total glucosinolate (expressed as butenyl isothiocyanate) and oxazolidine-2-thione contents of untreated and microwave-treated rapeseed meals used in feeding trials were 9.43, 2.93, 3.06 and 2.22 mg/g, respectively. The results of feeding trials are summarized in Table V. The poor results given by the untreated rapeseed must be attributed to the high glucosinolate content of this meal accompanied by thioglucoside glucohydrolase activity in the meal. Also, there could be heat sensitive nonglucosinolate substances (12,14) in the meal contributing to the low weight gains. Microwave treatment of rapeseed improved the palatability and nutritional value of meal, although weight gains were much below those of mice receiving the casein diet. The increased thyroid weights of the mice receiving the rapeseed meals suggest that both the untreated and the microwave-treated rapeseed meals were yielding goitrogenic glucosinolate hydrolytic products upon consumption. Although microwave treatment of rapeseed inactivated the endogenous myrosinase it appears that microflora of the intestinal tract of mice were able to effect hydrolysis of glucosinolates and produce goitrin in amounts similar to those produced from untreated meal.

The results of this work suggest that a 1.5 min microwave-heat treatment of dehulled rapeseed at initial moisture levels of 10 and 13% offers an alternative to the commercial dry heat treatment for inactivation of the endogenous thioglucoside glucohydrolase (EC 3.2.3.1) or myrosinase.

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TABLE V

Nutritional Evaluation of Untreated and Microwave-treated Tower Meals in Mice

Dietary preparation	Average weight gain (g) in 15 days	Average feed consumption (g)	Average thyroid weight (mg/100 g body weight)
Casein + 3.2% DL-methionine	11.2	85	9.3
Untreated Tower meal	-4.1	41	21.5
Microwave-treated Tower meal	3.8	69	35.1

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Chemical Investigation of the Seeds of *Brassica oleracea* Var. *Acephala*

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ABSTRACT

Fatty acid composition of the seed fat of *Brassica oleracea* Var. *acephala* (Cruciferae) has been determined. Erucic acid has been found to be the major component followed by linoleic, oleic, linolenic, arachidic and palmitic acids. Traces of stearic and eicosenoic acids have also been detected. The unsaponifiable matter contained β-sitosterol, and defatted seeds showed the presence of sucrose.

INTRODUCTION

Brassica oleracea Var. *acephala* – commonly known as "Kale" (1) – is a tall pot herb with curled leaves, grown in Assam, Bombay, Baroda, and Kashmir. The fatty acid composition of its seeds has not been worked out so far. Earlier detailed analyses of some allied species have been made (2), and the occurrence of eicosenoic acid in the seed fat has been confirmed (3).

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EXPERIMENTAL

About 300 g of the seeds were partially ground and extracted for 16 hr with petroleum ether (40-60 C) in a soxhlet. Removal of the solvent gave a yellow fixed oil in 6.5% yield. The physical constants of the oil were determined (Table I). It was saponified, and the mixed acids so

TABLE I

Physicochemical Properties of the Oil

Determination	Value
Specific gravity at 20 C	0.9010
Refractive index at 23 C	1.4741
Optical rotation at 23 C	-0.4'
Acid value	2.1
Iodine value	61.2
Saponification value	123.06
Acetyl value	89.8
Unsaponifiable matter	1.6%

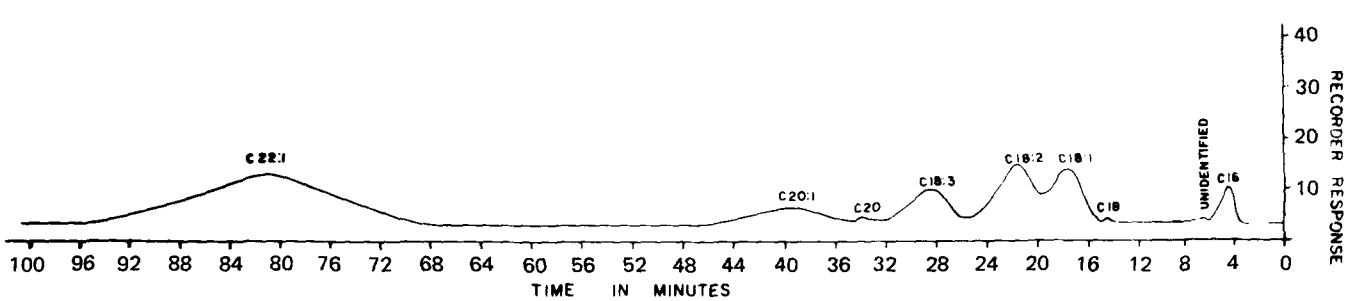

FIG. 1. Gas liquid chromatogram of the methyl esters of mixed acids on Reoplex column.