Diffusion Extraction of Rapeseed Glucosinolates With Ethanolic Sodium Hydroxide

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ABSTRACT

Glucosinolates are readily diffused from whole rapeseed by repeated extractions with ethanolic sodium hydroxide. Four 2 hr extractions or six 1 hr extractions were most efficient for the diffusion of glucosinolates with minimum losses of other seed constituents. The ethanolic sodium hydroxide treatment inhibited myrosinase activity and lowered sulfur concentration in the oil. The oil from treated seed gave a higher hydrogenation number than the oil from untreated seed. The oil content of rapeseed was not substantially affected by the diffusion extraction process. Loss of diffused solids was ca. 15%, and loss of nitrogen did not exceed 10%. Most of the nitrogen diffused from the seed was nonprotein nitrogen. The principal disadvantages of the ethanolic sodium hydroxide procedure were reduced solubility of the rapeseed proteins and the higher fiber levels in the meals. The treated seed may be suitable, after partial dehulling or air classification, for the preparation of protein concentrates.

INTRODUCTION

Rapeseed meal contains 35-40% protein (1) and has a well-balanced amino acid composition (2). The meal contains a substantial amount of basic and sulfur amino acids (3,4) and is therefore suitable to complement cereals and many legumes that are deficient in these amino acids. The amount of rapeseed meal in livestock rations is limited by glucosinolates which, on hydrolysis by myrosinase (thioglucosidase), yield toxic isothiocyanates, 5-vinyloxazolidine-2-thione (OZT), and organic nitriles (5,6). Several procedures have been reported in the literature (7-10) for the removal of these compounds from the meal so as to extend its use in feed and food products. Early studies by Bell and Belzile (7) employed various treatments of the meal. Some of these treatments inactivated myrosinase while others substantially reduced glucosinolates content. Catalytic decomposition of glucosinolates into organic nitriles and their subsequent removal by steam stripping removed toxic products except hydroxy-nitrile that remained in the meal (8). Ballester et al. (9) developed a water washing procedure that left 16% of the OZT and 23% of the isothiocyanates in the meal. These procedures do not completely remove the toxic factors from the meal, are not adaptable to commercial processing, or have resulted in excessive protein losses and a reduced protein quality (3,7).

Attempts have also been made to remove glucosinolates from whole or ground rapeseed. After inactivation of myrosinase by boiling, complete extraction of glucosinolates from ground rapeseed was achieved with an aqueous solvent (11). This process resulted in substantial losses of oil and protein. To overcome these losses Sosulski et al. (12) investigated diffusion extraction of glucosinolates from intact rapeseed. While diffusion extraction of rapeseed with 0.01 N sodium hydroxide was effective in the production of glucosinolate-free meal, the oil contained 80 ppm sulfur. Although such oil may be suitable for use as a cooking oil, the high sulfur content would make it undesirable for hydrogenation in the manufacture of shortening and margarine which use a substantial quantity of Canadian rapeseed oil (13). Reynolds and Youngs (14) have suggested that sulfur in rapeseed oil is mainly derived from the organic sulfur present in isothiocyanates and OZT, which are soluble in lipid solvents (6). Therefore a high sulfur content in the oil obtained from sodium hydroxide-treated rapeseed indicated substantial enzymic hydrolysis of glucosinolates during the diffusion extraction procedure. Inhibition of myrosinase during this process should prevent such hydrolysis, and as a consequence sulfur content of the oil would be reduced. Boiling the seed before diffusion extraction or the use of highly alkaline solvents were effective in controlling the myrosinase activity and sulfur level in the oil (12). However disintegration of the seed and adverse effects on oil and protein quality were potential hazards associated with these methods of myrosinase inactivation.

The objectives of the present study were to develop a process which would completely inactivate myrosinase during the diffusion extraction of glucosinolates from intact rapeseed. The effects of ethanol in the aqueous alkaline solvent on myrosinase inhibition and diffusion extraction of glucosinolates were investigated. The influence of the ethanolic sodium hydroxide solvent on oil yield and quality, meal composition and protein solubility are reported.

EXPERIMENTAL PROCEDURES

Diffusion Extraction of Rapeseed

Two varieties of rapeseed, Target *(B. napus L.)* and Echo *(B. campestris L.)* of the 1970 crop were used in the study. A weighed quantity of the seed (usually 20 g) was extracted in screw-cap polythene bottles with 10 volumes of ethanolic sodium hydroxide (0.01 N sodium hydroxide and 50% v/v ethanol, pH 12.0) for 8 hr with a change of solvent every 2 hr (4 x 2) or for 6 hr with a change of solvent every hour (6 x 1). The extraction was carried out in a shaking water-bath at 70 C. Target and Echo were also extracted for 4 hr (2 x 2), under identical conditions, with 0.01 N sodium hydroxide (pH, 11.6) alone or with 0.01 N sodium hydroxide after immersing the seed (in cloth bags) in boiling water for 2 min to inactivate myrosinase (15).

To determine the loss of protein nitrogen on diffusion extraction, ethanolic sodium hydroxide extracts were pooled and reduced in volume on a rotary evaporator. An aliquot of the extract was precipitated by adding an equal volume of 10% w/v trichloroacetic acid (TCA). After allowing to stand at room temperature for 30 min, the precipitated proteins were removed by centrifugation (10,000 x g; 20 min). An aliquot of the supernatant was taken for the determination of total nitrogen (16).

The extracted seed was rinsed with distilled water and dried for 2 hr at 60 C in a forced-draft oven. The loss in weight of treated and dried seed was taken as diffused solids. Oil was extracted from the seed by homogenizing with petroleum ether (Skellysolve F) in a Waring blendor, and the homogenate was filtered. This method of extracting oil was satisfactory, as the meal contained less than 2% residual oil as determined in the soxhlet. The oil was desolventized in a rotary evaporator. The oil-free meal was dried, until solvent-free, at room temperature (25 C)

Oil Analyses

Sulfur in the oil was determined by a procedure

TABLE I

Some Characteristics of Oil and Meal of Untreated and Treated Target and Echo Rapeseed[a]

Characteristic	Treatments							
				NaOH and Ethanol				
	Untreated	NaOH	Boiling and NaOH	2 x 0 hr	2 x 2 hr	3 x 2 hr	4 x 2 hr	6 x 1 hr
1. Oil								
Target								
Sulfur, ppm	4.5	73.0	16.5	---	5.1	---	4.1	5.2
Hydrogenation number ($\Delta RIX10^4$)	58.0	0.0	34.5	---	52.0	---	60.0	63.0
Free fatty acids, %	0.23	0.26	0.23	---	0.06	---	0.07	0.06
Echo								
Sulfur, ppm	2.3	71.0	12.0	---	5.6	---	3.7	4.8
Hydrogenation number ($\Delta RIX10^4$)	63.0	0.0	38.0	---	68.0	---	74.0	73.0
Free fatty acids, %	0.08	0.11	0.12	---	0.05	---	0.06	0.04
2. Glucosinolates, mg/g meal								
Target								
BI[b]	3.23	---	---	1.21	0.60	0.26	0.02	0.00
PI[c]	1.24	---	---	0.52	0.31	0.12	0.01	0.00
OZT[d]	10.90	---	---	3.40	2.40	1.30	0.45	0.00
Echo								
BI[b]	3.89	---	---	1.38	1.00	0.52	0.10	0.04
PI[c]	2.72	---	---	0.90	0.88	0.38	0.10	0.06
OZT[d]	6.10	---	---	2.90	1.00	1.10	0.00	0.00
3. Diffusable solids (DS) and meal composition, %								
Target								
DS	0.0	---	---	---	12.0	---	14.0	15.2
Oil yield	39.1	---	---	---	38.2	---	44.0	43.5
Protein	41.7	---	---	---	45.0	---	44.0	45.8
Fiber	10.8	---	---	---	12.5	---	17.9	18.3
Ash	7.2	---	---	---	7.1	---	7.8	9.1
Echo								
DS	0.0	---	---	---	12.5	---	13.5	14.2
Oil yield	38.2	---	---	---	40.8	---	43.4	44.3
Protein	43.3	---	---	---	45.1	---	47.8	48.0
Fiber	11.9	---	---	---	12.3	---	16.0	16.8
Ash	6.3	---	---	---	6.7	---	7.5	8.7
4. Solubility of meal proteins								
Target								
Alkali-soluble N, %	79.1	60.2	38.4	---	26.8	---	18.4	14.2
Isolate N, mg[e]	25.0	23.2	14.3	---	12.3	---	7.5	5.1
Recovery, %	40.3	33.3	18.6	---	18.3	---	13.0	8.8
Echo								
Alkali-soluble N, %	82.3	63.0	50.0	---	31.8	---	17.1	13.3
Isolate N, mg[e]	27.9	22.1	17.1	---	15.4	---	7.1	4.9
Recovery, %	45.0	29.9	23.4	---	23.0	---	11.6	8.2

[a]Results reported are averages of duplicate and triplicate determinations.

[b]3-Butenylisothiocyanate.

[c]4-Pentenylisothiocyanate.

[d]5-Vinyloxazolidine-2-thione.

[e]Per gram meal nitrogen.

described previously (12). The densitometer readings were converted to ppm sulfur by referring to a standard curve prepared with butyl isothiocyanate. Free fatty acids were determined on a 2.0-5.0 g oil samples by the official method (17) except that the indicator used was aniline blue.

Hydrogenation of Oil

Hydrogenation rates of the oil samples were determined as described by Reynolds and Youngs (14). Crude oil (30 g) was heated to 65 C and refined by adding 1.8 ml of 9.5% w/v sodium hydroxide. After stirring at that temperature for 15 min, the oil was centrifuged to remove the solid fraction. The supernatant fraction was decanted, heated to 110 C, bleached with 2% special Filtrol by stirring for 30 min, and then filtered through Celite. An aliquot (5 ml) of the refined and bleached oil was mixed with 36 mg nickel catalyst in a stainless steel tube and hydrogenated at 185 C for 30 min at a hydrogen pressure of 20 psi. The tubes were vigorously shaken during the hydrogenation process. The difference in refractive index (ΔRI) of unhydrogenated and hydrogenated oil was taken as the degree of hydrogenation and is reported as a whole number. The refractive index of oil samples was read in a Bausch and Lomb refractometer equilibrated to 60 C.

Meal Analyses

Protein (N x 6.25), ether extract, crude fiber and ash contents of untreated and treated meal samples were determined by the official methods (16).

Protein Solubility

One gram of each meal was extracted with 25 ml of 0.2% w/v sodium hydroxide, pH 12.0, by shaking for 1 hr in a wrist-arm shaker. The insoluble materials were removed by centrifugation (10,000 x g; 10 min). An aliquot of the supernatant was taken for the determination of alkali-soluble nitrogen (16); the rest was precipitated by slowly adding ca. 0.1 N hydrochloric acid to pH 4.2-4.5. The precipitate (isolate) was removed by centrifugation (10,000 x g; 30 min), and an aliquot of the supernatant was taken again to determine nitrogen not precipitated by the acid. The yield of isolate nitrogen is expressed as a percentage of original meal nitrogen.

TABLE II

Nitrogen Loss From Target and Echo Rapeseed During Diffusion Extraction With Ethanolic Sodium Hydroxide for 4 x 2 or 6 x 1 Hours

Variety	Seed N,[a] mg	Extract N, mg		N Loss, %		Nonprotein N in extracts, %	
		4 x 2 hr	6 x 1 hr	4 x 1 hr	6 x 1 hr	4 x 2 hr	6 x 1 hr
Target	882	96.7	99.0	9.1	8.9	73.0	86.6
Echo	860	90.4	99.7	9.5	8.6	63.1	74.1

[a]In 20 g seed.

Glucosinolates

Butenyl isothiocyanate (BI), pentenyl isothiocyanate (PI), and OZT contents of untreated and diffusion extracted meals were determined by the methods of Youngs and Wetter (18).

RESULTS

Quality of Oil and Meal

Some characteristics of oil and meal of untreated (control) and treated Target and Echo rapeseed are given in Table I. The sulfur contents of oil from untreated seed in the two varieties was between 2-5 ppm and showed little variation between a number of analyses of oil samples prepared separately. The hydrogenation number of oil from untreated Target was 58 and that of Echo 63; these values are means of three independent determinations. In both the varieties the oil from sodium hydroxide-treated seed contained over 70 ppm sulfur, and these oils would not hydrogenate, probably because of the poisonous effect of sulfur on the nickel catalyst. When the seed was first immersed in boiling water for 2 min to inactive myrosinase and then diffusion-extracted with sodium hydroxide under identical conditions, the sulfur content of the oil was reduced from 70 to 12-17 ppm, and its hydrogenation number rose to 36. The incorporation of 50% ethanol into the alkaline solvent reduced the sulfur levels in the oil to those of the oils from untreated seed. Under these solvent conditions the hydrogenation number of the 2 x 2 hr extraction treatment was slightly below the control value in Target but was higher in Echo. Extending the diffusion extraction to 4 x 2 hr or 6 x 1 hr resulted in oils that had better hydrogenation properties than the control oils, especially in Echo variety. Oil from ethanolic sodium hydroxide-treated seed was slightly greenish-yellow in color compared to the intense yellow color of the oil from the untreated seed; but, after refining and bleaching during the hydrogenation process, no physical differences in appearance, odor or viscosity of oils from treated and untreated seed were noticeable.

The concentrations of free fatty acids in oil from untreated Target and Echo were low and varied significantly between the varieties. Oil from untreated Target seed contained three times more free fatty acids than that of Echo. Free fatty acid concentrations were not reduced in either of the varieties by treatment of the seed either with sodium hydroxide or by immersion of the seed in boiling water followed by sodium hydroxide extraction. However ethanolic sodium hydroxide treatment considerably reduced the free fatty acid content, especially with Target, and only traces were present in the oil from both the treated seeds.

Glucosinolate contents of meals from untreated Target and Echo and those of meals from seed treated with ethanolic sodium hydroxide for different times are given in Table I. The meals were analyzed after every 2 hr of seed treatment to show the progressive removal of glucosinolates during the extraction procedure. After only 2 hr of treatment more than half of isothiocyanates or OZT were removed from the seed. After 4 x 2 hr treatment only small quantities of these compounds were found in the meals. More frequent solvent changes totally removed glucosinolates in 6 hr from Target, though trace amounts of BI and PI still remained in Echo meal.

Proximate Composition of Meals

Meals from ethanolic sodium hydroxide-treated seed were enriched in protein content, ash, fiber (Table I), and were lighter in appearance because of the removal of pigments and other low-molecular weight compounds from the seed. In Target total diffused solids was 14-15% for the 6 x 1 or 4 x 2 hr treatments, but the Echo sample was somewhat lower in losses of solids. This may account for the slightly higher protein and lower fiber levels in the Echo meal, but the total increase in protein content of both the varieties was nearly the same. The meals from treated seed contained ca. 16% fiber calculated on moisture and fat-free basis compared to 11-12% in the meals from untreated seed. The fiber content of the meals increased relatively little on treating the seed with ethanolic sodium hydroxide for 2 x 2 hr, but it increased sharply on treatment of the seed for 4 x 2 hr or 6 x 1 hr. The oil content of untreated Target and Echo varieties was 38-39%. The ethanolic sodium hydroxide-treated seed contained, on the average, 3-5% more oil than the untreated seed. This change in oil content resulted from the loss of diffused solids from the treated seed.

Solubility of Meal Proteins

The meals from untreated seed contained 79.1-82.3% alkali-soluble nitrogen (Table I). After isoelectric precipitation of the alkali-soluble proteins with dilute hydrochloric acid, 40-45% of the meal nitrogen was recovered in the isolates. Aqueous treatments of the seed affected the alkaline-solubility of the proteins, and hence nitrogen recovery was only 30-33% in seed treated with sodium hydroxide. When the seed was first immersed in boiling water for 2 min and then extracted with sodium hydroxide, nitrogen recovery was further reduced. Therefore boiling of seed even for a short length of time partially denatured proteins and affected their alkali solubility. Ethanolic sodium hydroxide treatment of the seed affected protein solubility even more than alkaline solvents alone and this affect was proportional to the length of the time the varieties were treated with the solvent. Six 1 hr extractions reduced protein solubility to only 13-14% and nitrogen recovery was only 8-9%.

The ethanolic sodium hydroxide extracts were highly colored and contained 9-10% of the seed nitrogen (Table II) and other compounds. A major portion of this nitrogen, 69% in Echo and 80% in Target, was nonprotein nitrogen which was not precipitated by trichloroacetic acid from the extracts. Extracts of variety Target contained 10% more nonprotein nitrogen than those of Echo, probably because of the original variation in nonprotein nitrogen contents of the varieties.

DISCUSSION

Two approaches are currently in use to remove the potentially toxic glucosinolates from rapeseed. Selective crossing of commercial varieties containing 5-6% glucosinolates with Bronowski which has only 0.5% glucosinolates (16) is employed to reduce these compounds from rapeseed. Second, chemical methods are used to remove glucosinolates from meal or whole seed after myrosinase inactivation. Chemical modifications of meal to reduce its glucosinolate content may cause unacceptable protein losses, as a quarter of meal proteins are water-soluble and more than two-thirds are salt-soluble (19). Removal of glucosinolates from whole seed offers the advantage that only small molecules may be diffused from the intact seed with only little loss of the large molecular weight proteins or lipids.

The procedure of Sosulski et al. (12) not only removes glucosinolates from the intact seed but also low-molecular weight compounds such as pigments, sugars, and amino acids, that react to form melanoids imparting a dark brown color to the meal (20). A major disadvantage of the method is the high sulfur content of oil from treated seed brought about by myrosinase hydrolysis of glucosinolates during the diffusion extraction process. Boiling of the seed for 3 min prior to alkaline extraction was reported to reduce the sulfur level in the oil to less than 10 ppm. However boiling of the seed is awkward and may affect protein and oil quality.

The experimental results reported here (Table I) show that the sulfur content of oil from treated seed could be reduced to the level of oil from untreated seed by diffusion extraction of rapeseed with ethanolic sodium hydroxide. This reduction in sulfur resulted from inhibition of myrosinase by ethanol. Consequently the oil from treated seed was comparable to that from untreated seed in appearance, odor, and its ease of hydrogenation. Oil from treated seed of both the varieties gave consistently higher hydrogenation numbers than oils from the treated seed. Low or almost negligible free fatty acids content of the oil from treated seed suggested that no hydrolysis of lipids or any substantial loss of oil occurred during the extraction procedure. The freeze-dried ethanolic sodium hydroxide extracts contained only 2-3% of ether-soluble materials. Because of the loss of diffused solids, the oil content of treated seed was 4-6% higher than that of the untreated seed (Table I). On storage of the oil from treated seed, especially with Target, a sediment appeared. This sediment was probably due to the precipitation of erucic acid containing glycerides.

In their detoxification procedure Eapen et al. (15) inactivated myrosinase by immersing the rapeseed in boiling water for 2 min. Although the oil obtained from the treated seed was light colored and contained low content of free fatty acids, the sulfur content and the hydrogenation properties of the oils were not reported. In our experiments similarly treated seeds (boiled but not diffusion extracted) gave oils which contained 5-8 ppm sulfur and indicated inactivation of myrosinase, in agreement with the authors. Such oil would be comparable to oil from untreated seed in its hydrogenating ease, as sulfur content is a fair indication of oil quality. Extraction of the wet-heat treated seed with sodium hydroxide to remove the intact glucosinolates showed the oil to contain 12-17 ppm sulfur. Such an increase in sulfur content and a correspondingly reduced hydrogenation number suggested that some myrosinase hydrolysis of glucosinolates occurred during extraction with sodium hydroxide. This agrees with previously reported heat-stability of myrosinase. Meals heated for 6 hr at 135 C still showed some enzyme activity (7). The present authors also noticed some enzyme activity in meals heated overnight at 110 C.

The meals from treated seed were light colored and almost totally free of glucosinolates (Table I). Only Echo meal contained trace amounts of BI and PI. These could probably be totally removed by treatment of the seed for an additional hour or by continuous extraction for a shorter time. Shorter extraction periods with more frequent solvent changes were more effective in the removal of glucosinolates than longer extraction periods.

Because low-molecular weight materials were removed from the seed during the extraction process, the protein content of meal obtained from treated seed was improved (Table I) and pproached that of soya meal. The loss of nitrogen was less than 10% of the original seed nitrogen (Table II) and was considerably lower than the protein loss reported by Tape et al. (11). Most of the nitrogen extracted from the seed was nonprotein nitrogen.

Principal disadvantage of the procedure is a reduced protein solubility of the meal because of partial denaturation (Table I). Diffusion extraction of the seed with sodium hydroxide, with or without prior boiling of the seed, also substantially reduced protein solubility. Probably the high temperature of diffusion extraction (70 C) contributed to partial denaturation of the seed protein. Because of the low protein solubility the treated seed is more suitable for the preparation of a protein concentrate rather than an isolate.

Rapeseed meal contains higher fiber contents than soya or sunflower meals (21). Ethanolic sodium hydroxide treatment of rapeseed increased fiber content of the meal to ca. 18% (Table I). Such a high fiber content is undesirable and needs to be reduced by suitable methods. Increases in fiber content of meal on treatment with different solvents have also been reported by other workers. The extracted meal obtained by Owen et al. (10) contained, on moisture and fat-free basis, 18.5% fiber; while rape meal obtained by Tape et al. (11) contained on the same basis over 30% fiber, which was reduced to about 8% by air classification of the meal.

Further evaluation of the method reported should be made to assess the feasibility of using the process on a commercial scale. In such an evaluation attention should be paid to the utilization or disposal of the effluent containing materials removed from the seed. It may be possible to use the procedure in a continuous process which will greatly reduce the time to remove glucosinolates from the seed.

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