Edible Protein Products From Cruciferae Seed Meals[1,2]

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Abstract

Rape, crambe and mustard seed are compared with respect to properties of the fixed oil, nature of the mustard oils and properties of the seed protein. Rapeseed (*Brassica campestris* and *B. napus*) has benefited greatly from plant breeding; the erucic acid content of the fixed oil and the level of glucosinolates can now be selected. Crambe seed (*Crambe abyssinica*), however, is still high in erucic acid and in glucosinolates. The glucosinolate patterns of the mustards are naturally simple. Procedures for preparing edible flours and isolates from rape, crambe and mustard seed are described. With mustard (*B. hirta, juncea* and *nigra*), the glucosinolate hydrolysis products are volatile and steam-stripping yields a bland flour. With rape and crambe seeds, the intact glucosinolates must be removed; aqueous extraction is practicable. The physical, chemical and nutritional properties of some of the meals, flours and isolates prepared from cruciferae seed are described.

Introduction

The Cruciferae family comprises 200 genera and 1600 species. Just as man has cultivated only 150 of the 200,000 species which comprise the division Angiosperm, so only a few genera of the Cruciferae family have been cultivated. In fact, confining discussion to the genus Brassica does not eliminate any oilseed of current value.

The genus Brassica provides edible leaves, edible tubers and oilseeds for use in feed, food or as condiments. For the purpose of this paper, attention will be focussed on only six species, the oil and protein contents of which are shown in Table I (1). The two rape species *Brassica campestris* (turnip rape, navette, colza or Polish-type rape) and *B. napus* (rape, colza or Argentine-type rape) are higher in oil content and lower in protein than the mustards or crambe.

There is a strong similarity among these species in their amino acid composition (Table II) (1). The amino acids contain reasonably high amounts of lysine and sulfur. The limiting essential amino acid is usually isoleucine. The high lysine levels mean that, when properly treated during processing, these cruciferae meals can provide protein of high quality. For example, rapeseed and mustard meals can be substituted for linseed meal, soybean meal or other vegetable protein supplements in livestock and poultry rations. The digestibility of its protein, however, is higher with ruminant than with nonruminant animals.

Crucifer seeds, however, contain glucosinolates which decompose by chemical or enzymatic reactions to yield a variety of products. Under the influence of myrosinase (thioglucoside glucohydrolase E.C.3.2.3.-1), the glucosinolates yield glucose, sulfate and a variety of mustard oils, depending on the composition of the parent glucosinolate (Table III). These sulfur-containing compounds are potent hydrogenation catalyst poisons if present in the oil and are the limiting factor in the use of these seeds as a source of protein for animal feed or human food.

Crambe Abyssinica

This oilseed crop is considered a rich source of oil for the oleochemical industry. Its fatty acids contain 56–62% erucic acid (C22, Δ13) with another 5% of its fatty acids of chain lengths longer than 18 carbon atoms.

Removal of the glucosinolates from crambe meal is a prerequisite to its use in feed or food. The initial work of the group at the USDA Northern Regional Research Laboratory, using an aqueous acetone extraction of the meal (2) led to the development of a procedure involving the autolysis of dehulled and defatted meal followed by extraction with aqueous acetone to remove the products of autolysis (3). Meals prepared in this way, and not heat-treated, had Protein Efficiency Ratios (PER) of 2.75 and 2.55 relative to casein at 2.50 (4). A procedure involving a sodium carbonate-steam treatment of full fat or solvent-extracted seed also improved the palatability and nutritive value of the meal (5). More recently, Van Etten et al. (6) showed that unhydrolyzed glucosinolates could be removed efficiently from intact crambe seeds by first blanching them and then leaching out the glucosinolates with hot water. Detoxified crambe meal has not yet been tested in foods for human consumption, however.

Mustard

Mustard seeds are characterized by the presence of only one glucosinolate, in contrast to the more complex situation in rape or crambe seeds. For example, *B. hirta* (white or yellow mustard) contains only sinalbin whereas *B. juncea* (brown or oriental mustard) and *B. nigra* (black mustard) contain mainly sinigrin. Thus, autolysis and steam-stripping would seem a practicable way to remove allylisothiocyanate from the seeds of the latter two species. Moreover, certain varieties of mustard appear to have very low contents of mustard oil.

Mustard seed contains 28% to 36% fixed oil. In *B. hirta* seed, the erucic acid content is about 40% while in *B. juncea* seed, it is only about 21% of the fatty acids, although some species (rugosa, integrifalia, and some Indian varieties) contain 45–48% erucic acid. The oil is generally prepared by autolysis

TABLE I

Protein and Oil Contents of Cruciferae Seed[a]

Genera and species	Oil content[c]	Protein content[b]	
		Whole seed[c]	Extracted meal[c]
Brassica campestris	42.7	23.8	41.5
B. napus	44.7	23.8	43.1
B. hirta	28.1	31.2	43.4
B. juncea	37.2	27.5	43.8
B. nigra	32.4	31.2	46.2
Crambe abyssinica	35.5	31.5	48.8

[a] Miller et al., J. Agr. Food Chem. 10: 426–430 (1962).
[b] Per cent N × 6.25.
[c] On dry basis.

[1] One of 9 papers presented at the Symposium, "Cruciferous Oilseeds," ISF-AOCS World Congress, Chicago, September 1970.
[2] Contribution No. 165 from the Food Research Institute, Canada Department of Agriculture, Ottawa, Canada.

TABLE II

Amino Acid Composition and Protein Score of Cruciferae Seed Meals[a,b]

Genera and species	Lysine	Methionine	Cystine	Isoleucine	Leucine	Phenylalanine	Tyrosine	Threonine	Value	MEAAi[c]	C.S.[d]	P.S.[e]
Brassica campestris	378	120	152	237	404	236	171	249	307	75	55	86
B. napus	364	111	152	228	395	221	164	240	301	74	52	82
B. hirta	362	97	124	207	412	233	206	171	300	71	48	73
B. juncea	335	104	159	236	395	240	167	251	296	74	52	82
B. nigra	274	94	148	235	391	234	165	232	288	71	45	68
Crambe abyssinica	314	106	177	239	382	246	172	255	287	74	52	77
Average value	319	92	134	222	372	235	178	235	289			

[a] Miller et al. J. Agr. Food Chem. 10: 426–430 (1962).
[b] Milligrams amino acid per gram of nitrogen.
[c] MEAAi, modified essential amino acid index.
[d] C.S., chemical score of the most limiting amino acid.
[e] P.S., protein score based on FAO provisional pattern (FAO Nutritional Study 16).

and steam-stripping of the volatiles. Its relatively long shelf life is attributed to the presence of tocopherols and other natural antioxidants.

In general, mustard protein is considered the equivalent of soy protein in nutritional value on the basis of its amino acid spectrum. Mustard flour contains no goitrogenic material nor does it appear to contain antigrowth factors or toxins. It could therefore serve as a useful protein supplement. Moreover, mustard flour has good functional properties. It stabilizes oil-in-water emulsions and milled, but unheated, mustard flour can absorb 1.5 times its weight of salad oil and 2.0 times its weight of water.

In North America, mustard flour, prepared by fine milling of the dehulled seed, is used essentially as a condiment-emulsifier. Mustard flour is prepared from either full-fat or partially defatted seed. It is commonly used at about the 1% w/w level in mayonnaise, salad dressing, sausages and luncheon meat. Its use in meat products is limited by United States law so that no more than 0.35% protein is added.

Recently, a procedure for the preparation of a bland protein meal from mustard was described by researchers at Mysore (7). Dehulled and defatted seed, in the form of a flour, was mixed with water (1:3 w/v) and the pH adjusted to 8.2. A paste of ground, cold-defatted, mustard seed was added as enzyme source and glucosinolate hydrolysis allowed to proceed for 4–5 hr. at room temperature. The liberated mustard oil was removed by steam distillation. The slurry was cooled and its pH adjusted to 5.0. The solid matter, which was collected by centrifugation, was washed once with water (1:3 w/v) to remove a bitter compound. The dried material contained 48% protein and 1.5% fiber.

Earlier, Mustakas et al. (8) developed a procedure for producing oil, meal and allyl isothiocyanate from oriental mustard on a pilot plant scale. The seed was cracked and flaked prior to being moistened to bring the water content up to 30%. The endogenous myrosinase was allowed to react for 1 hr at 128 F after which the meal was heated to about 200 F. Sparge steam was introduced to help the volatile mustard oil distill. After air-drying, the mustard oil-free meal was rerolled, hexane-extracted, desolventized and steam-stripped. The final product contained only 0.004% allyl isothiocyanate. Its nitrogen solubility index (3.4) was low but more moderate processing conditions have improved it (G.C. Mustakas, private communication).

TABLE III

Glucosinolates in Domesticated Crucifer Plants[a]

Plant	Glucosinolate	Organic radical
Brassica campestris	Gluconapin[b]	3-butenyl-
	Progoitrin[b]	(R)-2 hydroxy-3-butenyl-
	Glucobrassicanapin	4-pentenyl-
	Glucoalyssin	4-methylsulfinybutyl-
	Glucoraphanin	5-methylsulfinylpentyl-
B. napus	Gluconapin[b]	3-butenyl-
	Progoitrin[b]	(R)-2 hydroxy-3-butenyl-
	Glucobrassicanapin	4-pentenyl-
	Gluconasturtiin	2-phenylethyl-
	Glucoiberin	3-methylsulfinylpropyl-
	Sinalbin	*p*-hydroxybenzyl-
B. hirta	Sinalbin	*p*-hydroxybenzyl-
B. juncea	Sinigrin	allyl-
B. nigra	Sinigrin	allyl-
Crambe abyssinica	epi-Progoitrin[b]	(S)-2 hydroxy-3-butenyl-
	Sinigrin	allyl-
	Gluconapin	3-butenyl-
	Gluconasturtiin	2-phenylethyl-

[a] VanEtten et al., J. Agr. Food Chem. 17: 483–491 (1969).
[b] Major glucosinolate.

Rape

Both *B. campestris* and *B. napus* have received considerable attention from plant breeders. Varieties have been developed that contain essentially no erucic acid and plant breeders are actively seeking varieties free of glucosinolates. The Polish variety Bronowski (*B. napus*) is one of these. The challenge now is to introduce these highly important characters into plants with the desired agronomic features. The prognosis is good.

In parallel research, attempts have been made to modify the glucosinolates chemically or to leach them out of the crushed seed. Early investigations (9–11) of steam-stripping, autoclaving and hot water extraction for detoxifying rapeseed meal were ineffective, however.

In 1967, the chemical decomposition of rapeseed meal glucosinolates using iron, copper or nickel salts was studied (12). However, of the decomposition products, the toxic and nonvolatile 1-cyano-2-hydroxy-3-butene remained with the meal.

In 1966, the Food Research Institute (FRI) of the Research Branch, Canada Department of Agriculture, started its investigation of the aqueous extraction of mustard oil glucosinolates from rapeseed. A wet heat treatment for myrosinase destruction was developed (13).

Subsequently, in 1968, the production from rapeseed of a light-colored, bland, defatted flour low in glucosinolates was shown to be feasible (14). A flow diagram (Fig. 1) shows how this product, rapeseed flow (RSF) was obtained. Aqueous extraction at ambient temperature (near 20 C) proved superior to extraction at higher temperatures in that comparable removal of toxic material was achieved with lower loss of solids. Air classification, the final step in the FRI procedure, yields two streams: RSF and rapeseed meal (RSM).

The properties of these products are compared in Table IV. The products are essentially identical with respect to content of mustard oils and oxazolidinethione but differ in other properties.

Yield data and reproducibility were also explored. Twelve replicates were used to determine standard deviation. The results (Table V) show that loss of

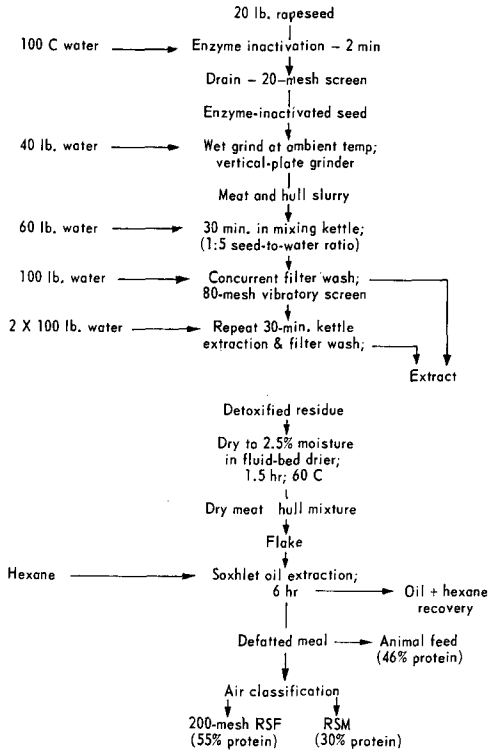

FIG. 1. Procedure for the preparation of nontoxic flour and meal from rapeseed (K.E. Eapen et al. JAOCS 45: 194–196 (1968).

solids due to aqueous extraction is high. This, plus an obvious need to conserve water, requires the substitution of a more sophisticated extraction procedure for the simple batch system now in effect.

Each phase of the FRI procedure was monitored by determining its effect on the nutritive value of the product produced at that stage (15). Rat-feeding tests were used to provide PER (16) using casein as internal standard. The PER value of rapeseed increased substantially after the wet heat inactivation of myrosinase and the removal of the glucosinolates by aqueous extraction. These results substantiate the need for both enzyme inactivation and aqueous extraction in the production of nontoxic products from rapeseed.

The PER values of three lots of rapeseed flour and meal prepared over a period of eight months (Table VI) show that RSF has a slightly higher PER and RSM a slightly lower PER value than casein. Differences between batches were not statistically significant.

Researchers at the University of Chile, in cooperation with the Department of Food Science and Technology at the University of California, have studied rapeseed meal (17) and its detoxification (18) over the past two years. Aqueous extraction at room temperature reduced the content of both isothiocyanates and oxazolidinethione but a "double water extraction" (one extraction lasting from 8–14 hr followed by a second of 1–3 hr duration) was considered to be the most satisfactory of the methods tried. In common with the results of the FRI research, these authors report increased palatability of the detoxified rapeseed meal.

TABLE V
Average Yields and Percent Removal of Solids at Each Step of Process[a]

Process step	Dry weight of solids (average of 12 lots), lb.	Standard deviation (12 lots)	Loss of solids from previous step, %
Echo seed	18.74	0.19	.....
Enzyme inactivated	18.49	0.51	1.3
Ground seed	18.25	0.14	1.3
Aqueous extracted	14.26	0.10	21.8
Defatted	7.79	0.18	45.7
Rapeseed flour	3.89	.....	.....
Rapeseed meal	3.89	.....	.....

a N.W. Tape et al. CIFT J., 3: 78–81, 1970.

In addition to measuring the chemical, physical, functional and nutritional properties of our rapeseed flour, we have investigated possible uses for it. Rapeseed flour has been incorporated into hamburger patties, cookies, noodles, doughnuts, cakes and bread. This work is quite recent and the statistical analysis of the taste panel assessments has not yet been completed. Qualitatively, the outlook is favorable.

An alternative to the removal of glucosinolates by aqueous extraction has been developed by Staron (19). Through the action of the fungus *Geotrichum candidum* on rapeseed presscake, the glucosinolates are destroyed and the rape protein made more soluble.

A certain interest has developed in the preparation of protein isolates from rapeseed. Between 1963 and 1967, Pokorny and colleagues published a series of 11 papers on the alkali extraction and acid precipitation of rapeseed protein (20,21). In this series, the effects of pH, concentration, solids-liquid ratios, temperature and contact time were established. A broad, low isoelectric point (pH 3.5) was encountered.

In 1968, Shaikh et al. in Pakistan (22), prepared a glucosinolate-free protein isolate from commercial rapeseed cake and studied its properties. Defatted meal was extracted with aqueous sodium hydroxide (pH 8–11) and precipitated at pH 4, 5 or 6; pH 10 for solution and pH 4 for precipitation proved most satisfactory.

This isolate, shown to be free of isothiocyanates, was used in nutritional studies with the original defatted meal as control. Fish flour and skim milk powder, alone and in equal parts with the rapeseed isolate, were also included in the test. All protein was fed at the 10% level. The Net Protein Utilization

TABLE IV
Proximate Composition and Mustard Oil Content of Rapeseed Flour and Meal[a]

Product, 1969	Composition, dry weight basis					Mustard oil	
	Protein,[b] %	Fat, %	Fiber, %	Ash, %	NFE, %	Isothio., mg/g	Oxazol., mg/g
RSF[c] (Jan)[d]	54.7	8.3	8.0	9.5	19.5	nil	0.002
RSF (Apr.)	54.9	9.1	5.9	7.8	22.3	Trace	0.003
RSF (Aug.)	52.1	10.9	8.8	9.8	18.4	Trace	0.003
RSM (Jan.)	34.2	4.3	30.2	4.7	26.6	nil	0.001
RSM (Apr.)	28.5	4.3	24.6	3.6	39.0	Trace	0.003
RSM (Aug.)	31.4	5.2	27.1	4.9	31.4	Trace	0.003

a N.W. Tape et al. CIFT J., 3: 78–81, 1970.
b N × 6.25.
c Abbreviations: RSF, rapeseed flour; RSM, rapeseed meal.

TABLE VI
Protein Efficiency Ratio (PER) Data for Rapeseed Flour and Meal Samples[a]

Product, 1969	PER[d]		Body weight gain (g/rat/ 28 days)	Protein intake (g/rat/ 28 days)
RSF[b] (Jan.)[c]	2.67	0.077	104	29.1
RSF (Apr.)	2.54	0.081	107	31.7
RSF (Aug.)	2.56	0.058	104	31.2
RSM (Jan.)	2.28	0.041	98	33.4
RSM (Apr.)	2.03	0.033	88	32.8
RSM (Aug.)	2.02	0.056	93	35.3

[a] N.W. Tape et al. CIFT J., in press.
[b] Abbreviations: see Table IV.
[c]
[d] Corrected value using casein as an internal standard with a PER of 2.50.

of the isolate was 69%, that of the original meal, 53%, and those of the 50:50 mixtures of isolate plus fish flour and isolate plus skim milk powder, as high as that of the reference substances (99% and 88%, respectively).

In 1969, Sosulski and Bakal (23) isolated protein from rapeseed, flax and sunflower meals and compared these isolates to soy protein isolates prepared in the same manner. Defatted meals were made on a laboratory scale and subjected to successive extraction using distilled water, 5% sodium chloride, 70% ethanol and 0.2% sodium hydroxide (Table VII). Varietal differences within a crop were noted. The water solubility of the proteins varied markedly. The protein from *B. campestris* seed was less soluble than that from *B. napus* seed and each less soluble than soy. The simpler solubility pattern of soy protein is also evident.

Korolczuk and Rutkowski have also studied the solubility patterns of the nitrogenous material in rapeseed meal (private communication). Through varying the pH of the aqueous extraction medium and the temperature of extraction, various fractions were obtained. Two major fractions were obtained at pH 4 (temperature independent) and pH 7.5 (temperature dependent).

TABLE VII
Peptization of Proteins in Soybean, Rapeseed, Flax and Sunflower Meals by the Osborne Series of Four Solvents[a]

Crop and variety	Per cent of total meal nitrogen soluble in				Per cent nitrogen in residue
	H_2O	5% NaCl	70% EtOH	0.2% NaOH	
Soybean, dehulled					
Portage	69.0	8.2	4.5	5.4	12.9
Altona	75.7	6.0	3.9	4.1	10.3
Rape					
Argentine	51.3	20.5	3.9	8.1	16.2
Target	50.6	20.5	4.0	9.1	15.8
Oro	48.4	22.4	3.3	8.5	17.4
Turnip rape					
Polish	44.6	24.0	4.1	6.3	21.0
Echo	44.5	25.0	4.4	6.6	19.5
Zero erucic	44.7	24.7	4.3	5.5	20.8
Flax					
Redwing	41.6	46.5	1.2	3.2	7.5
Redwood	52.1	34.4	2.0	3.5	8.0
Noralta	43.6	43.6	1.2	2.9	8.7
Sunflower, dehulled					
Commander	19.2	59.8	3.1	11.5	6.4
Advent	16.9	60.2	3.5	11.6	7.8
Peredovik	22.9	50.9	4.1	11.9	10.2

[a] F.W. Sosulski and A. Bakal, CIFT J. 2: 28–32 (1969).

At the FRI we study the proteins, carbohydrates, lipids, tannins, polyphenols, glucosinolates and minor components of the seed. We are concerned with the color that develops in rapeseed meal and flour, particularly under alkaline conditions. We have reduced the intensity of color in our rapeseed protein isolates to our satisfaction. Recently, Sosulski has prepared light tan isolates as has Chichester (private communications).

The most extensive reports on rape protein are those of Finlayson et al. (24). These researchers extracted the salt soluble proteins from eight varieties of rapeseed using 0.01 M sodium pyrophosphate (pH 7.0) and 1.0 M sodium chloride and separated them into a number of components. The pyrophosphate extract contained two major proteins, a neutral 12 S protein and a basic 1.7 S protein. Together, they constituted 30% of the nitrogen in the extract. The 1.0 M sodium chloride extract also contained a 12 S protein which accounted for 21% of the nitrogen in the extract and which resembled the 12 S protein in the pyrophosphate extract. The 12 S and the 1.7 S proteins from the different varieties had similar electrophoretic and chromatographic behavior but differed in amino acid composition, especially with respect to sulfur-containing amino acids. Porath and colleagues at the University of Upsala have also fractionated rapeseed protein. Their work, however, has not yet been published.

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