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A Process for Producing Nontoxic Rapeseed Protein Isolate and an Acceptable Feed By-Product

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ABSTRACT

Pilot-plant studies have indicated that a light tan, bland, nontoxic rapeseed protein isolate can be produced commercially from rapeseed presscake meal for use in supplementation of foods or feeds. The simple extraction and washing procedures produce a product of approximately 84% protein containing 0 to 0.22 mg. of oxazolidinethione per g., with isothiocyanates completely eliminated. The original rapeseed presscake meal contained 7.5 mg. oxazolidinethione and 4.9 mg. isothiocyanate per g. of meal. The residual solids after protein extraction can still be used as a high-protein supplement of greatly reduced thioglucoside content, thus enhancing its value as a by-product feed supplement for nonruminants.

Rapeseed meal is well known to be a virtually untapped source of oilseed protein. Its liberal use as a feed is barred by the presence of thioglucosides (more recently referred to as glucosinolates) which when hydrolyzed by the enzyme myrosinase produce isothiocyanates. The isothiocyanates and their transformation products such as oxazolidinethione, formed by cyclization of beta-hydroxy isothiocyanate, are potent antithyroid substances producing goiters in both man and animals (1).

Rapeseed is the fifth-largest oilseed crop (5 million metric tons) in the world, but in Chile is the foremost, yielding some 47,000 metric tons of extracted presscake annually (2). This represents about 17,000 tons of extractable protein, now used inefficiently because of the toxicity of its accompanying thioglucoside.

Much recent effort toward detoxifying the meal has had varied success. Canadian workers such as Eapen et al. (3) and Sosulski and Bakal (4) have demonstrated the water-solubility of the thioglucoside content as offering the most promising method of elimination. Ballester et al. (5), of Chile, developed a water-washing technique for meal detoxification that is effective in eliminating

thioglucosides. More recently (while the present work was being carried out), Eapen et al. (3) published an account of a water-extraction procedure of rapeseed to yield a bland, high-protein, toxin-free flour.

This investigation was undertaken to produce, with pilot-plant equipment, rapeseed meal protein isolate in reasonable quantity, of high purity, and by a method easily and economically adaptable to a commercial operation.

MATERIALS AND METHODS

Centrifuges: Broadbent, Type 86, Rigid-Bearing Centrifuge, Huddersfield, England, and International Centrifuge, Model CS, Needham Heights, Mass.

Special filter bag for Broadbent Type 86 Centrifuge: light cotton canvas 76x76/inch^2 thread count.

pH meter: Fisher Accumet, Model 210.

Mixer: Hy Speed Mixer, Type 4, Allsop Engineering Corp., Milldale, Conn.

Colloid mill: A. Mannesmann Emulsor, Type 201F, Remsheid, Germany.

Spray dryer: A/S Niro Atomizer No. 1191, Copenhagen, Denmark.

Rapeseed presscake meal: purchased from Coprona y Cia., Santiago, Chile. A mixture of Regina, Matador, and Norin 16 (all *Brassica napus* varieties). The meal, a fair representation of this mill's commercial output, was extracted by an expeller-solvent (hexane) process with controlled temperatures not exceeding 110°C. at any stage.

Calcium carbonate, hydrochloric acid, and sodium hydroxide were all analytical grade. Baker's.

Sodium chloride: common table salt purchased in the local markets.

Proximate analyses were determined according to AOAC Methods of Analysis (6).

Oxazolidinethione and isothiocyanates were determined according to the methods of Appelqvist and Josefsson (7). Original thioglucosides were determined according to McGhee et al. (8).

Five kilos of the rapeseed presscake meal was slurried with a minimum of 25 liters of potable tapwater (ambient temperature), and particle size was reduced by passing the mixture twice through the colloid mill at maximum setting (Table I). After 1 hr. this slurry was made to a working volume of 50 liters with tapwater, and 3 kg. of sodium chloride was added. The resulting mixture was stirred mechanically for an additional hour. Temperatures were those of the pilot plant (5° to 10°C.). Earlier exploratory runs under controlled temperature conditions (60°C.), giving only slightly greater extraction, were abandoned as economically unrewarding.

The final slurry was transferred from the stirring tank into the basket-type centrifuge fitted with a filtration bag, and filtered at 1,300 r.p.m. (505xg). Transfer was done uniformly over a period of 30 min. The filtered extract was collected at the liquid outlet of the centrifuge and placed in precipitation vats. The extracted solids remaining in the filter bag were sprayed (in spurts of 5 to 6 sec.) with water from the internal washing system of the centrifuge at intervals during centrifugation and twice at the end of the collection. Spray volume was precalibrated at 6.1 liters per min. Samples of the extracted rapeseed meal were taken for analysis.

The solution in the precipitation tanks was adjusted from a normal pH 5.9 to pH 2.5 with hydrochloric acid to precipitate the proteins, which were allowed to

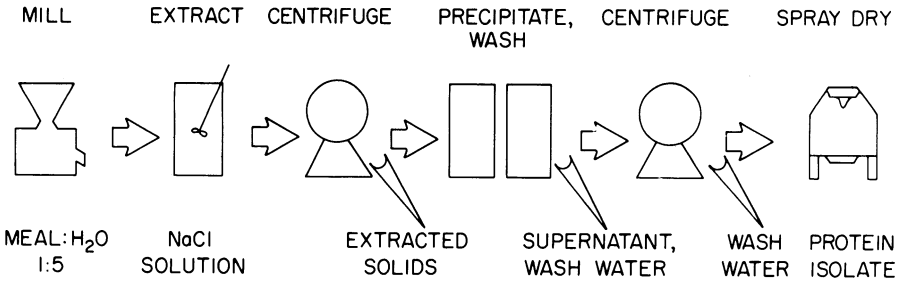

Fig. 1. Process flow diagram.

settle. The supernatant fluid was siphoned off after 2 to 3 hr., and discarded. The protein precipitate was then remixed with a volume of fresh water equal to that discarded. This procedure was repeated three more times. After the fourth washing, calcium carbonate was added at the rate of 1 g. per 10 liters of precipitated proteins (about 2% solids), and the solution was adjusted to pH 5 with 10% sodium hydroxide. This procedure appears to increase the protein curd strength and precipitation rate. The treated protein was then brought to its original volume with tapwater and allowed to settle. The supernatant was siphoned off, and the precipitated protein was then centrifuged for 10 min. at 2,000 r.p.m. (350xg) while being washed with water equal in volume to the precipitated curd. This procedure was repeated three times.

The washed precipitate was collected and slurried with enough water to produce a solution of 7% solids for spray drying. Mechanical stirring was needed at this point to prevent lumping.

This final slurry was then spray-dried. The collected solids, being hygroscopic, were transferred to sealed containers. Figure 1 summarizes the process.

RESULTS

In preliminary trials, grinding the meal prior to extraction increased protein yield some 10% with the grinding periods used. Reduction in particle size is shown in Table I. This procedure also makes easier the handling and pumping of the slurry, besides increasing surface exposure to the extracting medium.

Table II shows changes in the proximate analyses, isothiocyanate, and oxazolidinethione of the extracted rapeseed meal solids. Both fiber and ash

TABLE I. PARTICLE SIZE[a] REDUCTION WITH COLLOID MILL

Mesh	Original %	2X Through Mill %
20	13.9	3.3
40	51.8	36.7
60	16.4	22.0
Pan	17.7	37.9
Total	99.8	99.9

[a]Determined 5 min. on Fisher-Wheeler sieve shaker with Fisher U.S. standard sieves, brass, 8 in. diam., 2 in. deep with indicated mesh sizes.

TABLE II. COMPOSITION COMPARISON OF PRE- AND POSTEXTRACTION MEALS[a]

Rapeseed Presscake Meal	Moisture %	Protein %	Ether Extract %	Crude Fiber %	Ash %	Oxazolidinethione mg./g.	Isothiocyanate mg./g.	Thioglucosides %
Original	9.28	35.87	1.13	13.33	6.55	7.52	4.86	3.61
Extracted	9.11	25.56	1.04	16.69	20.80[b]	0.75	0	0.97

[a]Reported on as-is basis (6), (7).

[b]Sodium chloride 14.7%.

TABLE III. SPRAY-DRYER CONDITIONS

External Factors	Feed rate	1.5 liter/hr. (gravity feed)
	Solids content	5.8 to 7%
	Plant temperature	8° to 10°C.
	Air pressure	5.8 – 6.0 k./cm.^{2}
	Heaters	3
	Air inlet temperature	175°C.
	Exhaust temperature	70°C.
	Exhaust setting	Maximum

TABLE IV. COMPOSITION COMPARISON OF PROTEIN BATCHES[a]

Run No.	Moisture %	Protein %	Ash %	Oxazolidinethione mg./g.	Isothiocyanate mg./g.
7–1	3.47	83.87	2.52	0.22	0
22–2	2.12	84.88	3.40	0	0
12–3	2.19	83.57	2.30	0	0

[a]Reported on as-is basis. Methods (6), (7), (8).

contents have relatively increased in the residual meal, through the reduction in protein and water-soluble carbohydrates. The sodium chloride retention in the extracted meal is a function of the final washing. In these trials no attempt was made to decrease this level, since the meal is considered of a secondary nature and its feeding properties have been thoroughly explored by Ballester and Yañez through three generations of laboratory rats (personal communication). Further washing would doubtless also decrease the already reduced oxazolidinethione residue.

Table III shows the spray-drying conditions in the Niro. The partial proximate analysis of the protein (Table IV) shows almost complete detoxification of the product as well as the uniform concentration of protein produced by the process. Color has been a light tan, with no variation. Taste is bland, with no distinguishing character or aftertaste.

The yield and loss data in Table V indicate extraction of about 40% of the protein theoretically available. The area of loss appears to be the technique employed in precipitating the extracted proteins, for both the free amino acids and the proteins soluble at the pH used are discarded. Average recovery of extracted

TABLE V. YIELD AND LOSS DATA, AVERAGE OF THREE RUNS

Total Protein in Original 5 kg.	Extracted	Super-natant	Protein Loss in Washes[a] 1	2	3	4	5[b]	Centrifuge Washes[c]	Net Yield
Grams 1,790	720	187	120	59	18	6	6	4	320
Percent of Theoretical Total 100	40.3	10.4	6.7	3.3	1.0	0.35	0.35	0.2	18

[a]Washing by settling and decantation.

[b]Washing with added calcium carbonate.

[c]Combination of three washes in centrifuge.

proteins to date is about 45%. However, our unreported biological data indicate that this method preserves protein quality and is in agreement with Drouliscos and Bowland's finding (9). In laboratory studies reported recently, Sosulski and Bakal (4) also note such a loss in their isoelectric precipitation of the protein of rapeseed, although their recovery percentage (25%) was greater than experienced in this investigation. Further work is indicated in this phase.

DISCUSSION

This method, as proposed, has been shown to be capable of yielding high-purity protein of good biological value (at least equal to that of soy protein isolate)[1] and yielding this protein consistently at 18% yield levels (Table V). Preliminary work did show that higher yields could be obtained with an alkaline extraction and subsequent protein precipitation at the several isoelectric points favorable to the different fractions (10) observed in rapeseed protein. Technical difficulty in adapting this more sophisticated procedure to a simple process ruled out its use. The foregoing observations are in agreement with findings of Eapen et al. (3) and Bhatty et al. (11).

Certain features of the process should be emphasized since they are critical to the efficiency of the operation. First, adjustment to pH 2.5 must be reasonably fast since the initial curd formed determines wash-tank holding time. Secondly, the calcium carbonate addition and subsequent pH adjustment to pH 5.0 carry the curd through the final three centrifuge washings. Finally, attention must be given to creating a smooth slurry prior to spray-drying, since at the solids level employed the centrifuged protein tends to agglomerate, creating problems at the spray-dryer head.

Rough cost estimates, toxicity trials, protein efficiency ratios, and postmortem examinations of sacrificed trial animals to date have all indicated the protein produced by this method to have a high potential for meeting human demands for new protein sources.

The greatly reduced levels of isothiocyanate and oxazolidinethione in the

[1]Protein evaluation ratio for our isolate was 1.86 vs. standard (NCR casein) of 2.7. Hegsted and Chang reported a protein evaluation ratio of 1.52 for soy protein isolate at 11.92% protein level diet. J. Nutr. 85: 159 (1965).

extracted meal would permit the use of higher levels of rapeseed meal in the feeding of poultry and swine. Current levels of feeding are generally limited to 10% or less of the total ration (10), with some doubt as to the eventual deposition of the toxic factors in eggs or tissues. Unreported data show a further fractionation of the meal to be a possibility, one fraction being a light-colored high-protein (40%) flour such as reported by Eapen et al. (3), and the other a darker, higher-fiber, lower-protein pulp.

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