EXTRACTION AND DETOXIFICATION OF RAPESEED PROTEINS

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While rapeseed oil has found wide application in food products, rapeseed meals are usually discounted as animal feeds and have not found application in human nutrition. Despite their excellent amino acid balance, rapeseed meal contains high levels of glucosinolates, crude fiber, dark pigments and strong flavors which are undesirable in food products. Unless myrosinase activity in the seed is controlled, the sulfur compounds will also contaminate the oil.

Numerous procedures have been devised for detoxification of the meal-steam stripping will remove the isothiocyanates but not the oxazolidinethione. Under high temperatures, metallic ions will degrade the glucosinolates into nitriles, but the hydroxy-nitrile that originates from oxazolidinethione is not steam volatile and exhibits greater toxicity. The glucosinolates can be washed from the meals by extraction with aqueous or organic solvents (Ballester et al.,1970) but long extraction times and high solids losses usually occur (Table 1). Tape et al. (1970) extracted glucosinolates from ground seed with two water-washings but the wet slurry required specialized driers and high losses of oil and protein have been reported (Kozlowska et al., 1972). The aqueous diffusion of intact seed controlled dry matter losses while removing a high proportion of glucosinolates, simple sugars, polyphenolic acids and other low molecular weight compounds (Sosulski et al., 1972). The intact seeds were readily dried before oil extraction, or the wet seeds could be dehulled by flaking, drying and air separation which fractionates hulls and meats quite efficiently. The principal disadvantages of the seed washing technique were long extraction times and large volumes of water required to maintain the diffusion gradient across the seed membranes. High solvent temperatures, high pH (pH 11) or ethanol in the aqueous solvent aided in inactivating myrosinase, glucosinolate removal and pigment extraction (Table 1).

Table 1. Efficiency of extraction procedures on glucosinolate removal, control of sulfur (S) in the oil and solids losses in the extracts from Brassica napus cv. Zephyr[1]

Extracted Product	Solvent	Aglucones in meal mg/g	S in oil ppm	Loss in Extract % Oil	Protein	Yield of Products % Oil	Meal
Fresh seed	-	13.2	100	-	-	38	60
Defatted meal	Water	Trace	-	-	8	-	44
Ground seed	Water	0.8	2	10	8	27	41
Intact seed	Water	0.2	27	0.1	3	37	48
Intact seed	Ethanol	0.2	0	0.2	3	37	49

[1]Abstracted from Kozlowska et al. (1972).

CONTINUOUS DIFFUSION EXTRACTION

The batch diffusion procedure, while very effective for the removal of glucosinolates, utilized seed to water ratios of 1:20, and the water was changed after each hour of the four-hour extraction (Table II). Clearly the large volumes of dilute extract containing glucosinolates, simple sugars and nitrogen compounds would constitute a serious pollution problem.

An extract drying or recycling operation would be very expensive.

Continuous extraction methods were then investigated as a means of reducing the solvent volumes and diffusion times. The continuous diffusions were conducted in a heated open vessel equipped with a stirrer and overflow valve. The initial seed to solvent ratio was 1:10, and fresh water

Table II. Residual glucosinolates in the meal after batch, continuous and countercurrent diffusion extraction of intact rapeseed (Brassica campestris cv. Span)

Extraction procedure and cultivar	Extraction conditions			Aglucones in the meal (mg/g)		
	Solvent volume	Temp. °C	Time min.	Isothiocyanates Butenyl	Isothiocyanates Pentenyl	Oxazolidine-thione
Untreated Span	---	---	---	3.6	2.0	2.0
Batch	80:1	60°	240	0.0	0.0	0.0
Continuous	45:1	80°	60	0.6	0.3	0.0
Continuous	30:1	90°	90	0.1	0.1	0.0
Countercurrent	5:1	90°	30	0.1	0.1	0.0

was introduced continuously during the course of the extraction. The diffusion process was found to be very temperature dependent. The previous temperatures of 50° to 60°C in the batch experiments were too low for rapid diffusion during the continuous procedure. However, all of the oxazolidinethione was diffused from Span rapeseed at 80°C in one hour (Table II). Unfortunately, the butenyl and pentenyl isothiocyanates were more difficult to remove, but 90°C proved to be adequate for their extraction. The design of the continuous extractor limited the reduction in solvent(tap water) volume below 30:1.

Sulfur levels in the oil were routinely measured in each of the water diffusion experiments. For diffusions at 50° to 60°C, the seeds were dipped in boiling water for 3 minutes to inactivate the enzyme and control the sulfur content of the oil to less than 10 ppm. The continuous diffusions at 80° to 90°C did not require a pretreatment to give high quality oils with 2 to 7 ppm of sulfur.

COUNTERCURRENT DIFFUSION SYSTEM

Subsequently, a batch countercurrent extraction system was investigated in which 4 or 5 large extractors are utilized simultaneously. While the seed remained in one extractor during the complete diffusion cycle, the extracts were transferred from tank to tank in a countercurrent rotation. Thus, fresh rapeseed came in contact with liquor or extract solution which had been used for three previous extractions and, after the fourth diffusion, passed to the driers or recycling operation (Table III). The same seed lot was then progressively diffused with extracts from the second and first extractions and, finally, with fresh water. Since all stages of extraction take place simultaneously in separate tanks, the total diffusion time was equivalent to that of a single extraction-20 to 30 minutes in the present study. This countercurrent system was found to be as effective as the continuous extraction in glucosinolate removal and had the very important advantage of using very low water to seed ratios (Table II). A 5:1 ratio was used in the initial experiment, but it appears even lower ratios were possible since the solids load in the extract or liquor was only 3 to 4%.

Table III. Ratios of fresh seed weight to extract volume during each stage of a four-stage countercurrent extraction of glucosinolates from rapeseed

Extractor	A	B	C	D
Extract in,ml	440	470	495	500
Seed wt,g	100	165	170	175
Extract out,ml	350	440	470	495
Seed wt,g	165	170	175	180
Liquor solids,g	12.3	6.2	2.6	0.8

The water requirements and utilization scheme for the batch countercurrent system of seed diffusion were as follows:

	Water/seed ratio
Total water use in the diffusion system	5/1
Liquor for recycling or evaporation-drying	3.5/1
Water in seed to be removed by drying	1/1
Evaporative losses in extractors, pumps, etc.	0.5/1
Fresh water requirement - if recycled	1.5/1
- if liquor dried	5/1

LOW GLUCOSINOLATE CULTIVARS

With the availability of genes for low glucosinolate content in Bronowski, several cultivars with reduced glucosinolate level have been developed in Brassica napus (Tower, Regent) and B. campestris (Candle). The oilseed meals from these cultivars have been found to be relatively safe for livestock feeds but the low levels of oxazolidinethione would be objectionable in a rapeseed flour intended for the food market.

Experiments were conducted to determine the lowest solvent to seed ratio (1:1, 2:1, 3:1), shortest time (15, 30, 60 min.), fewest number of extractions (1,2,3) and optimum temperature (60°, 70°, 80°, 90°C) required to detoxify the low glucosinolate varieties (Bronowski and Tower). Extractions were conducted under alkaline pH to inactivate the enzyme, myrosinase, and avoid sulfur contamination in the oil. After diffusion the hot extracts of glucosinolates and dark pigments were drained from the seeds through cheesecloth before drying the glucosinolate-free seeds and extracting the oil. A portion of the wet seeds were passed between rolls to press out the meats, and the hulls were blown off after seed drying but before oil extraction. The defatted product in the latter case would be a "protein concentrate" because both fibrous material and some water-soluble constituents were removed.

Preliminary experiments demonstrated that at least 3 parts of dilute alkaline solvent per volume of seeds were required to diffuse significant amounts of glucosinolates from the seed but larger volumes failed to improve extraction efficiency. Similar studies showed that the previous countercurrent extraction process was unnecessary for the low glucosinolate cultivars and, as shown in Table IV, two batch extractions of 30 minutes each were effective for glucosinolate removal at high (80°C) temperatures. At low temperatures, up to five extractions were not satisfactory for complete diffusion of these sulfur compounds from the seed.

Table IV. Residual glucosinolates in the meal or concentrate after batch diffusion extraction of whole rapeseed under a range of extraction conditions

Cultivar and extraction procedure	Extraction conditions			Aglucones in the meal (mg/g)		
	Solvent volume	Temp °C	Time min	Isothiocyanates Butenyl	Pentenyl	Oxazolid-inethione
Bronowski						
Untreated meal	-	-	-	0.43	0.32	0.16
DE concentrate	2(3:1)	22	2(30)	0.42	0.36	0.35
DE concentrate	2(3:1)	80	2(30)	0.06	0.05	0.00
Tower						
Untreated meal	-	-	-	0.28	trace	0.80
Untreated flour	-	-	-	0.25	trace	0.94
DE meal	2(3:1)	50	2(30)	0.14	trace	0.78
DE concentrate	2(3:1)	50	2(30)	0.15	trace	0.74
DE meal	2(3:1)	80	2(30)	0.04	0.00	0.00
DE concentrate	2(3:1)	80	2(30)	0.06	0.00	0.00

The extracts from the two-stage diffusion process were freeze-dried and weighed to show that only 5.7% of the original seed solids were lost in the extraction. As compared to the meal, the untreated flour contained 8 percentage units more protein and 10 percentage units less crude fiber (Table V). However, the ash content of the flour was not reduced by dehulling. The protein percentage in DE-80°C concentrate was less than 60%, as compared to 37% in the rapeseed meal. Due to the loss of soluble constituents, the DE concentrate was higher in crude fiber and ash than untreated flour. A protein isolate was made from the DE concentrate by alkali extraction and isoelectric precipitation to show that the ash constituents are firmly bound to the protein in rapeseed.

Table V. Proximate composition of Tower meal and meal products (%, dry basis)

Tower product	Yield % of seed	Protein (N x 6.25)	Fat	Fiber	Ash
Untreated meal	57.0	36.7	2.4	14.6	7.3
Untreated flour	36.5	44.9	1.1	4.8	8.4
DE-80°C concentrate	29.0	58.7	2.2	6.1	9.1
Protein isolate	14.5	82.6	0.6	0.1	9.9

It appeared that the present glucosinolate removal procedure could be readily incorporated into a commercial oil extraction plant. The economic feasibility of the process would depend on the potential food uses and functional properties of the rapeseed concentrate. The wet dehulling step was effective in hull removal but, unfortunately, the hulls contained 24% oil which represented one-tenth of the original seed oil. The preferred procedure would be to extract all of the oil from the dried seeds after diffusion extraction, and fractionate the hulls from the flour as a post-extraction procedure.

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