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### EFFECT OF PROCESSING ON THE CHEMICAL COMPOSITION OF RAPESEED MEAL

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#### INTRODUCTION

Industrial processing causes changes in the natural properties of agricultural products. These changes may be positive or negative. The stronger and longer the action, the greater will be the changes. Thermal processes are among the important factors influencing the quality of the products, while mechanical factors or the humidity and the solvent are less significant.

The effect of the above-mentioned factors on the quality of solvent-extracted meal as a feed ingredient is particularly complicated. On the one hand, processing is inevitably accompanied by changes which are frequently detrimental to the most valuable component, the protein. On the other hand, we are intentionally applying relatively drastic conditions in order to remove physiologically hazardous lipid components which often occur in seeds. These two conflicting tendencies are subject of this paper.

#### PROTEINS AND THERMAL PROCESSING CONDITIONS

Protein is the most important component of rapeseed meal. Its character and level are the main measure when evaluating the feed quality of the meal. Apart from small quantities of vitamins, the proteins are the substances most responsive to temperature effects.

The protein content of rapeseed meal amounts to  $36 \pm 2\%$  (Table I). Its content depends only to a limited extent on processing technology, but its quality is influenced greatly by the thermal conditions which may significantly decrease its nutritional value.

The investigation of the solubility of nitrogenous substances in a) water, and b) in aqueous solutions of electrolytes represents one of the most popular methods of evaluating the changes resulting from heat treatment. The results of the analysis of protein solubility indicate that the continuous extraction methods are the mildest (Table II). The content of insoluble nitrogenous substances in meals produced by these

methods amounted to little over 30% while in batch extractions they amounted to more than 50%. Larger disproportions can often be observed when comparing the amount of nitrogenous substances soluble in water and in NaCl, which are the protein fractions most easily assimilated by non-ruminants (26).

TABLE I

PROTEIN CONTENT OF DIFFERENT OILSEED MEALS

	Protein in Meal
	%
Peanut Meal	45 - 55
Soybean Meal	40 - 50
Cottonseed Meal	35 - 45
Sunflowerseed Meal	40 - 50
Rapeseed and Other Cruciferous Meals	30 - 40

TABLE II

CONTENT OF N-SOLUBLE COMPOUNDS (%) IN RAPESEED MEALS EXTRACTED BY THE BATCH AND CONTINUOUS SYSTEM (26)

N-Soluble Compounds in		Batch Extractor (17 Samples)	Continuous Extractor (32 Samples)
Water	Range Average	13.5-18.8 14.9	21.6-28.2 25.9
10% NaCl	Range Average	9.8-17.5 12.4	25.8-29.8 27.6
0.2% NaOH	Range Average	15.1-22.2 18.7	12.4-15.9 14.6
Insoluble	Range Average	48.0-60.7 53.5	27.0-35.5 31.9

These observations can be explained on the basis of causes occurring during processing. In the batch method the desolventizing conditions favor protein denaturation, i.e. at a temperature of 120°C in humid environment. However, in a continuous extraction process desolventization generally does not exceed 110°C and occurs at a lower level of humidity.

The range of values obtained for the different processes also permits the conclusion that the thermal conditions are more stable in the continuous than in the batch extraction process, thus giving a more uniform product. This refers particularly to the desolventizing and drying conditions of the meal, since the extraction conditions are usually several degrees below the boiling point of the solvent and similar for all methods.

The threshold for considerably decreasing the solubility of the nitrogenous substances of rapeseed meal under plant conditions lies at 115°C and above 30 minutes. It must also be stated that the temperature level is most important regarding protein denaturation, while the time of heating is only of secondary significance.

The changes occurring in the solubility of nitrogenous substances in the meal upon evaporation, i.e. under conditions close to those of desolventization by the batch method, follow a similar pattern (Table III). The higher decrease of protein solubility under those conditions agrees with the generally accepted views of the greater sensitivity of proteins to denaturation in a moist than in a dry environment.

TABLE III(26)

CONTENT OF WATER N-SOLUBLE (% N) COMPOUNDS  
IN DRY HEATED, AND WET STEAMED RAPESEED MEAL

Rapeseed Meal	Content of Water N-Soluble Compounds
Cold Extracted	38.2%
<u>After:</u> Dry Heating 30 mins., 105°C	31.6%
Wet Steaming 30 mins., 105°C	20.8%

Sometimes the effect of heating on the protein value of the meal is determined by the change in digestibility. This type of evaluation is more significant in the feeding of non-ruminants and is less important in ruminants. Table IV indicates that this determination gives higher values for protein meals produced by the continuous than by the batch extraction method. This is probably due to the larger formation of melanine compounds during the batch, which are more resistant to enzyme action.

The above-mentioned results were to some extent confirmed upon the determination(13) of the alpha-amino nitrogen content of meals, which was higher in the continuous extraction than in the batch extraction samples.

The protein quality of a meal depends first of all on the amino acid composition, and in this respect rapeseed meal is equal or even surpasses that of soybean protein.

Clandinin(8) as well as Blaizot(6) indicated that the higher the temperature used during processing and especially during cooking, the lower is the nutritional value of the meal, something

TABLE IV

PROTEIN DIGESTIBILITY OF RAPESEED MEALS  
EXTRACTED IN THE COLD BY THE BATCH AND THE CONTINUOUS SYSTEM (25)

Extraction System	Protein Digestibility %N		
	Pepsin	Tripsin	Pepsin + Tripsin
Laboratory, cold, Soxhlet	86.7-87.4	83.1-89.8	90.1-91.9
Industrial continuous	81.3-83.4	78.4-81.6	86.2-88.5
Industrial, batch	73.3-77.6	69.9-75.2	78.4-82.9

that is usually expressed by the decrease in available lysine. For example, when straight expeller processing, which was accompanied by relatively high temperatures, was replaced or complemented by solvent extraction, the lysine content in the meals increased by 20-30%.

When investigating the batch extraction process(12), it was found that considerable losses occurred through the liberation of such amino acids as tryptophan, methionine, threonine and serine as well as slight losses of lysine, cysteine, tryptophan and phenylalanine. In the case of the continuous extraction process the losses of bound amino acids are indistinguishable, while the losses through liberation were considerably lower (Table V).

TABLE V

CONTENT OF ALPHA-AMINO NITROGEN IN RAPESEED MEAL EXTRACTED BY THE BATCH AND THE CONTINUOUS SYSTEM (13)

Extraction Type	Alpha-Amino Nitrogen %N
Laboratory, Cold, Soxhlet	1.6-1.9
Industrial, Continuous	1.3-1.5
Industrial, Batch	0.7-1.5

In the protein evaluation of meals and feed undergoing thermal processing, the determination of free eta-amino groups of lysine plays an important role (27). This determines the available lysine and indicates a relationship to the extent of influence of the thermal processing conditions (Table VI).

TABLE VI

CONTENT OF AVAILABLE LYSINE IN RAPESEED MEAL EXTRACTED BY THE BATCH AND THE CONTINUOUS SYSTEM (27)

Extraction Type	Available Lysine	
	g/16 g N	loss %
Laboratory, Cold, Soxhlet	3.43	0.0
Industrial, Continuous	3.34	2.7
Industrial, Batch	3.15	8.2
Meal Heated 1h/135°C	2.85	16.9

These results clearly indicate that the protein in rapeseed meal undergoes considerable changes upon processing, and their significance depends to a large extent on the temperature.

CHANGES IN SACCHARIDES AND THE NON-ENZYMATIC BROWNING REACTION

The content of crude fiber, starch and pentosans in rapeseed meal does not change during processing, but the monosaccharide content, which plays an important role in the non-enzymatic browning reaction, changes considerably. The complex character of this process has not yet been fully explained (13) and some authors interpret its course simply on the basis of the change in the colour of the meal. Some information may also be obtained through the analysis of some of the components of the meal.

The decrease in the amount of reducing sugars in the meal before and after inversion is larger when the processing temperature increases (Table VII). A similar trend was shown in the decreasing content of liberated saccharides. Thus, it can be concluded that the more drastic the processing conditions, the greater will be the losses in mono- and oligosaccharides.

TABLE VII

CONTENT OF REDUCING SUGARS IN RAPESEED MEAL  
EXTRACTED BY THE BATCH AND CONTINUOUS SYSTEM (13)

Extraction Type	Reducing Sugars (%)	
	Before	After
	Inversion	
Laboratory, cold, Soxhlet	3.7 - 4.4	10.6 - 11.5
Industrial, Continuous	3.4 - 3.9	9.6 - 10.9
Industrial, Batch	2.3 - 2.7	8.1 - 8.6

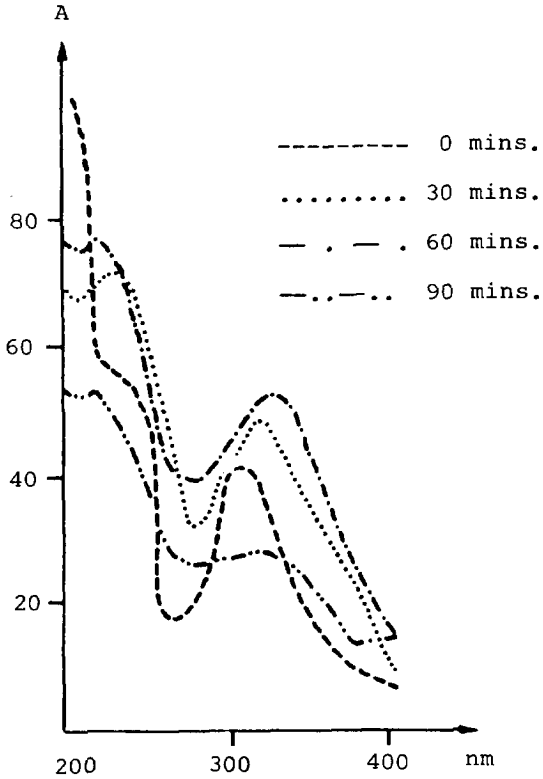


FIGURE I (24)

SPECTRA (200-400 nm) OF ETHANOL (80%)  
EXTRACTS FROM RAPESEED MEAL TOASTED AT 105°C FOR DIFFERENT  
PERIODS OF TIME (MINS.)

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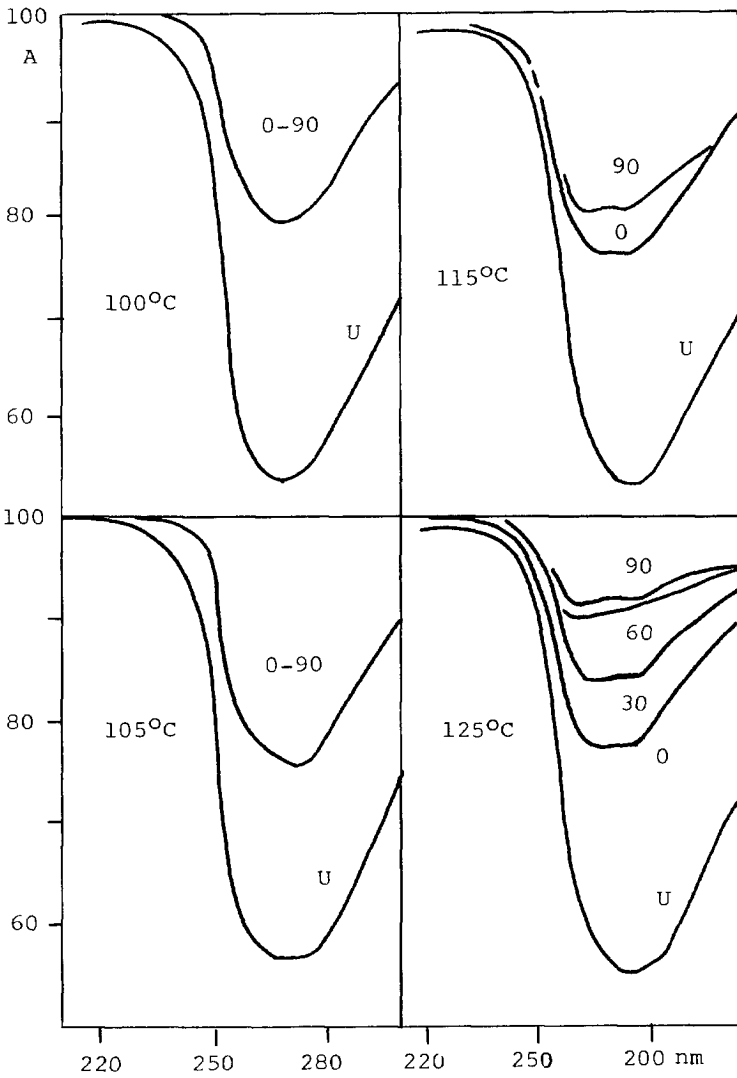


FIGURE II (24)

INFLUENCE OF TEMPERATURE AND TIME OF TOASTING ON THE UV SPECTRA (220-300 nm) OF ETHANOL (80%) EXTRACTS FROM RAPESEED MEAL.

A = Absorbance %, U = Untreated, 0-90 = Time in Mins.



The observed decrease in the amount of reducing saccharides as well as the decrease in lysine and  $\epsilon$ -amino nitrogen, may be linked with the darker colour of the meal, provided that they result from the formation of high-molecular weight brown Maillard compounds. At a temperature of 105-110°C only relatively slight changes occur, and only the drying at temperatures of 115°C and higher have considerable effect on the development of the dark colour of the meal, which is intensified with the time of exposure to these temperatures. When the drying (calcination) temperature amounts to 135°C, far-reaching changes are brought about, which cannot be distinguished visually based on the time of exposure.

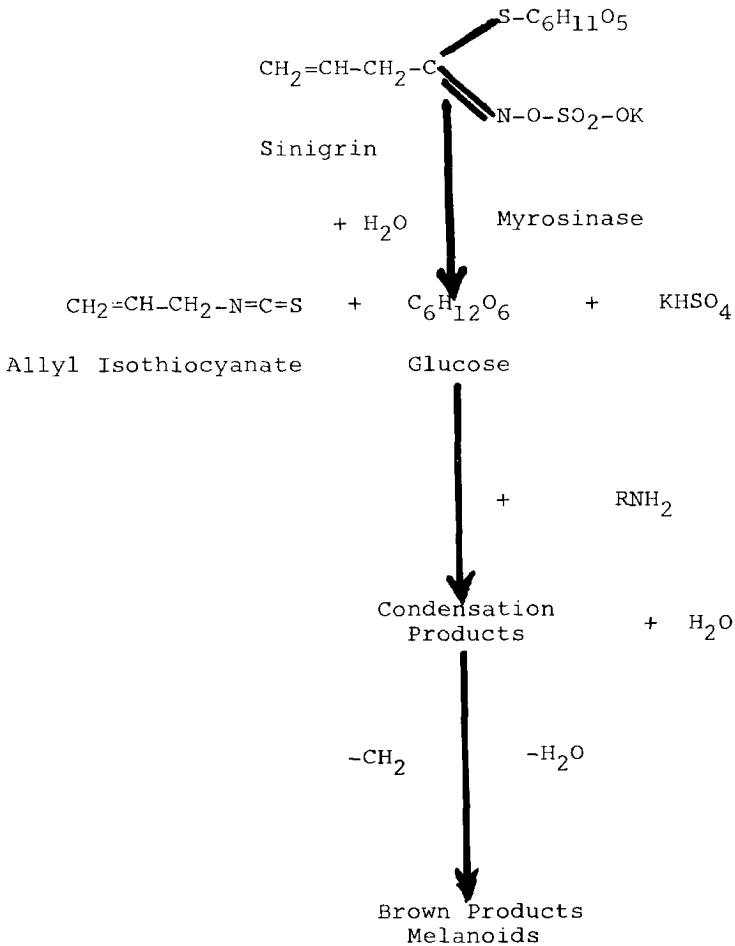
The changes in the colour of the meal are visible, and they are undoubtedly related to the thermal conditions of processing. They are not only the result of the non-enzymatic browning reaction but also of the complete decomposition of pigments, such as flavonoids, carotenoids and chlorophylls at 115°C.

The spectrographic analysis of the ethanol extracts of rapeseed meal indicates that the quantity of thermal decomposition products of saccharides increases considerably just above 105°C, showing the increase in absorption of the extract at 220 nm (hydroxymethyl-furfural) and at 280 nm (anhydro sugars). Substances produced upon heating increase the absorption in the range of 250-300 nm (Figures I and II) corresponding to condensation products formed upon heating of a glucose solution with l-glutamin or d,l-lysine (282-285 nm). However, the presence of peaks at 210 nm proves the presence of peptide rings and indicates a connection with the toasting process (20, 21). Further calcination shows the increase of absorption at 380 nm, which is typical for melanoid compounds.

The presence of glucose (24) which is liberated from thioglucosides, easily results in reactions with amino acids producing dark compounds (Figure III). It was also indicated that there

FIGURE III

FORMATION OF MAILLARD COMPOUNDS BY  
DECOMPOSITION OF GLUCOSINOLATES



is a possibility of forming coloured products by the reaction of sinigrin with amino acids at relatively low temperatures. It has been assumed (15) that in this case a reaction occurs involving the oxime or isothiocyanide group of the sinigrin molecule.

The changes in the components of rapeseed meal discussed above and having rather negative effects on its nutritional value, are the result of the conditions under which the oil is extracted.

The need for a meal possessing the highest biological value led to a number of studies aiming at the development of processes removing the goitrogenic thioglucosides.

#### CHANGES IN LIPIDS, VITAMINS AND MINERAL SUBSTANCES

The changes in the lipid composition of the meal are greater the more exhaustive the extent of extraction. The phospholipids and lipoproteins are substances which are not easily extracted and may be tightly bound to the seed tissue. Among others, this is the reason why the fatty acids of the residual lipids contain less erucic, eicosenoic and linolenic acids and more of the other acids than are found in typical rapeseed oil (Table VIII).

TABLE VIII (36)

#### COMPOSITION OF MAIN FATTY ACIDS IN RESIDUAL FATS AND RAPESEED OIL

Fatty Acid	Residual Fat %	Rapeseed Oil %
16: 0	5.8 - 7.3	3 - 4
16: 1	0.4 - 1.5	0.3
18: 0	0.9 - 1.1	1.0
18: 1	16.2 - 16.7	11 - 15
18: 2	18.8 - 23.2	11 - 13
18: 3	8.0 - 9.9	6 - 10
20: 0	0.4 - 1.5	1.0
20: 1	9.1 - 14.0	8 - 12
22: 1	24.3 - 36.8	47 - 53

The ash content, i.e. mineral matter, in relation to dry non-fatty material, does not undergo any changes during the production process(36). Only the iron content doubled from 16 mg/100 g dry non-fatty matter to 30 mg/100 g as a result of corrosion and deterioration of the production machinery(13).

All vitamins, except for tocopherol, are relatively unaffected by the production process, and we may assume that they do not undergo significant changes during the crushing process. However, we have not yet gathered sufficient data to fully substantiate this opinion.

The above-mentioned changes in the meal are the result of processing conditions. On the basis of its chemical composition it can be said that rapeseed meal may be classified as a very valuable protein for animal feeding. It does not produce such valuable feedstuffs as soybean or sunflowerseed meals.

#### REMOVAL OF GOITROGENIC SUBSTANCES

When asserting that rapeseed meal may be classified as a very valuable protein feed, there is one restriction.

Meals of the plants of the Cruciferae family contain goitrogenic substances, which have a negative effect on their nutritional properties. The presence of anti-nutritional substances has also been found in other oilseed meals, e.g., aflatoxins in peanut meal, gossypol in cottonseed meal and several different compounds such as trypsin inhibitors, haemagglutins, anti-coagulants, saponin, lipoxydase, as well as goitrogenic, ricketogenic and diuretic factors in soybean meal. They have a detrimental effect on the quality of the products. The demand for high-quality rapeseed meal resulted in studies of technological processes which would remove the glucosinolates and their derivatives. In most cases it is possible to neutralize the anti-nutritional factors by changing the thermal conditions of crushing technology.

The most important problem, namely the removal of goitrogenic compounds from Cruciferae meals, however, turned out to be more complicated than anticipated.

Good results were obtained only in the debittering of mustard-seed meal, which contained only isothiocyanates and no other goitrogens. A group of American researchers led by Mustakas(19) developed an effective procedure for the manufacture of a meal completely free of isothiocyanates.

Investigations into the improvement of rapeseed meal were based on the following concepts:

- 1) The removal of volatile substances by distillation or toasting, without prior breakdown of glucosinolates.

- 2) The hydrolysis of glucosinolates and the removal of liberated products by distillation.
- 3) The extraction of glucosinolates and of their products of hydrolysis.
- 4) The microbiological and chemical destruction of goitrogenic substances.
- 5) The de-activation of myrosinase in the raw material to be processed.

Steam distillation of the isothiocyanates, which are the main cause of the pungent flavour of the meal, has so far given the relatively best results. An early example of this technique is the method of André (2) who detoxified rapeseed meal in this manner first in France about 20 years ago.

Steam treatment has a strong influence on the reduction of the isothiocyanate and oxazolidinethione content of the meal (Table IX). The meal obtained is sweet and has lower but, nevertheless, distinctly goitrogenic properties. The negative aspect of the process is the considerable reduction in protein quality.

Efforts to obtain positive results in the removal of goitrogens have also been made by toasting, which had been found useful in soybean processing. It had been stated that temperature has a considerable influence on oxazolidinethione (OZT) content, but in practice the isothiocyanates proved to be highly temperature resistant despite their volatility. According to Altschul (1) and Frolich (10) temperatures in the range of 105°C do not destroy OZT. Only dry calcination at 130°C and 150°C will bring about an effective decrease in the goitrogenic properties of rapeseed meals. We have found (28) that calcination of the meal at 120°C destroys 80% of the OZT. Calcination at 130°C leaves only traces of goitrogenic substances.

Investigations of industrial toasting in the toaster-desolventizer, which were carried out with Polish as well as Canadian meals, indicated a decrease of more than 50% of the ITC and OZT content (24). However, a decrease of about one-third of the amount of soluble proteins was observed at the same time, and the more drastic the process the greater will be the decrease. Under our processing conditions only a slight decrease in digestible protein (79-86%) compared with the original material (approx. 88%) was observed (17).

Other methods, such as heating the meal with ammonia and iron salts, have not shown practical results, and have so far not

TABLE IX (23)

CONTENT OF ITC, OZT, SCN (IN MG/G) AND SOLUBLE PROTEINS (%N) IN  
RAPESEED MEAL COOKED AT DIFFERENT TEMPERATURES AND TIMES

Heating temp.	time	I T C		O Z T		S C N		SOLUBLE PROTEIN	
		mg/g	%	mg/g	%	mg/g	%	% N	%
no	0	4.2	100	13.5	100	0.4		28.4	100
105°C	30 min	3.9	93	11.2	84	0.2		23.6	84
	60	3.5	84	9.7	72	0.2		21.9	77
	90	3.3	78	6.4	47	0.1		20.1	71
115°C	30	2.9	69	6.7	50	0.1		19.2	68
	60	2.6	62	5.2	39	0.1		16.6	59
	90	2.3	55	4.6	34	0.1		7.9	28
125°C	30	2.2	52	4.8	36	0.1		13.1	47
	60	2.0	48	3.2	24	0.1		6.1	21
	90	1.8	43	3.0	22	0.1		3.5	12
135°C	30	1.4	33	1.4	10	0.1		5.6	20
	60	0.8	19	0.7	6	0		2.8	10
	90	0.6	14	0.6	5	0		0	0

been widely used. Attempts have also been made to hydrolyze the glucosinolates at elevated temperatures with weak solutions of sulfuric acid, but apart from removing the glucosinolates, this method caused also a considerable destruction of lysine (30).

An example for this process is the method of Goering (11), where not only the free isothiocyanates but also their glucosidic compounds are removed from the meals. To achieve this, the meal is first macerated in the presence of myrosinase before carrying out the distillation. The fact that the rapeseed meal contains active myrosinase able to decompose glucosinolate into goitrin and isothiocyanate, could be used in this process. It is destroyed only at 80°C. Since the procedure takes 2-3 hours, it becomes impractical in a continuous extraction process.

Another approach to solve this problem is the attempt to destroy the myrosinase before the isothiocyanate and goitrogen are liberated from the glucosinolate. This would allow to obtain a meal completely free of isothiocyanate and goitrogen. When using this meal in feeding, it should be considered that goitrogenic substances could be liberated from the glucosinolate under the influence of enzymes present in the alimentary canal, and a goitrogenic effect could appear.

Other studies did not show that glucosinolates of rapeseed meal evaporate together with the vapor after the action of hydrolytic enzymes in the alimentary tract of swine and poultry. However, although the goitrogenic substances can be removed from rapeseed meal after several hours of distillation, it is not used in practice, since it is too expensive and also considerably lowers the protein value.

Considerable amounts of goitrogenic substances can be removed through the application of organic solvents such as ethanol, methanol and acetone. The greater the hydrating capacity of the solvent, the greater will be the amount of goitrogens removed (Table X). A solvent concentration of 50% achieved the highest removal of isothiocyanate and other goitrogenic substances. Lower concentrations caused technological difficulties during the drying and solvent regeneration stage.

The removal of over 90% of the glucosinolate is possible when seed is extracted which has been treated with water at 80-90°C. The seed pulp is extracted with water at 60-90°C, and this is followed by the extraction of the oil from the pulp with a solvent. The recovered meal contained more than 50% of protein and slight amounts of glucosinolates (35). Glucosinolates can be easily extracted with 75-80% methanol, and the loss of proteinaceous material is reduced through extraction at pH 3.5-4.5 or 8-9 (34). Extraction with wet acetone or its

mixture with hexane, and preceded by maceration at 25-27°C, has also been reported (31).

TABLE X (23)

REMOVAL OF ITC AND OZT FROM RAPESEED MEAL  
BY METHYL ALCOHOL SOLUTIONS

Rapeseed Meal	ITC		OZT	
	mg/g	%	mg/g	%
Untreated	7.6	100	8.4	100
Extracted by:				
MeOH 100%	6.3	81	7.5	89
90%	4.7	62	6.9	82
80%	4.3	56	5.4	64
70%	3.6	47	4.6	55
60%	2.9	38	4.0	48
50%	2.6	35	3.5	42
Water	2.2	29	1.0	12

Among the various processes aiming to remove toxic substances from the meal (Table XI), the extraction procedures give products having considerably higher biological values of the proteins (7), and they are the best. Unfortunately, the costs and the difficulties of scaling up to plant-size and continuous operations prevent their practical application.

TABLE XI (7)

BIOLOGICAL VALUE (THOMAS MITCHELL) AND NPU  
(MILLER BENDER) OF RAPESEED MEAL PROTEIN  
AFTER EXTRACTION BY METHANOL AND ETHANOL

	BV	NPU
Untreated	65.2	62.3
Extracted by 75% Methanol	76.9	72.2
75% Ethanol	76.6	69.3

A most interesting new method of biological detoxification has been reported at this Conference by Staron (29). This method



is based on the maceration of rapeseed meal with *Geotrichum candidum*. This would hydrolyze the thioglucosides accompanied by the partial destruction of the isothiocyanates and the liberation of soluble toxic proteins. The recovered protein meal when fed to mice showed a greater gain in weight than soybean or peanut meals, and the aqueous fermentation solution contained all the toxic substances.

The difficulties encountered when trying to debitter rapeseed meal has led some experts to the opinion (22) that in practice it is impossible to remove the goitrogenic substances.

The lack of satisfactory results obtained when trying to use these methods to remove goitrogenic substances and the lowering of the protein quality, have led to another approach namely to the selection by the plant breeder of rapeseed varieties with a low pro-goitrin content. The advantages of this approach are substantiated by the known differences in the content of goitrogenic substances found in rape and turnip-rape (Table XII) or by the absence of OZT in white mustard.

TABLE XII

ITC AND OZT CONTENT IN RAPESEED - (mg/g nfs.)

	ITC 3-butenyl	ITC 4-pentenyl	OZT	Total
<u>Winter Rape:</u>				
Gluzower Oelquel	3.97	0.73	9.77	14.47
<u>Spring Rape:</u>				
Tanka	3.35	0.66	11.02	15.03
Nugget	1.95	0.32	9.96	12.23
Bronowski	0.29	0.14	0.37	0.70
<u>Turnip Rape:</u>				
Polish Rape	1.98	5.19	1.85	9.02
Sarson	8.48	0.00	0.00	8.48

The results of investigations carried out in Poland (18), Canada (32) and Sweden (3), which are based mainly on spring Bronowski rapeseed, indicated the possibility of obtaining satisfactory results by this approach as well as species free of glucosinolates. It can be assumed that this method

will provide the best way of obtaining rapeseed meal of the highest feed value, surpassing that of meals derived from other oilseeds.

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1) QUESTION: In your paper you referred to sodium hydroxide extraction, and I was wondering what tests you did to measure the effect of the sodium hydroxide on the protein, on the amino-acid content or on the growth?

ANSWER: (Professor A. Rutkowski)

You asked about the measure of protein quality, and the extraction of protein meals. In our laboratories we measure the effect on the amount of soluble protein. This is expressed as ratio of total nitrogen to protein nitrogen. The extraction of amino acid during this process was investigated on the basis of the amount available lysine. We have also investigated the effect on the amount of rare amino acids, but I have not given these results since there was no significant difference affecting the quality of rapeseed meal.