SOME TECHNOLOGICAL ASPECTS OF THE ISOLATION OF RAPESEED PROTEIN

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Introduction

In the course of the search for inexpensive and readily available raw materials for the production of protein concentrates and isolates, attention has only recently been given to rapeseed. Rapeseed protein is characterized by a relatively high content of "sulphur amino acids" and lysine. Studies on the quality of rapeseed protein have shown that it is characterized by a high biological value. This finding aroused particular interest in rapeseed protein in countries such as Canada, Poland, Sweden, Germany and France where rapeseed is cultivated as a basic oil crop.

Direct utilization in animal or human nutrition of rapeseed protein in the form of seed pulp or fat-free rapeseed flour is difficulties due to the presence of glucosinolates. These are precursors of the goitrogenic 5-vinyl-1,3-oxazolidine-2-thione and isothiocyanates.

While it is possible to reduce the relatively high level of cellulose in rapeseed by sifting or winnowing away the husks in an air stream, the removal of glucosinolates proves more difficult. Toasting, which has been successfully used to overcome the antitryptic agent in soybean, has also been used to improve the quality of extracted rapeseed meal. A decrease in the amount of goitrogenic compounds (RUTKOWSKI et al., 1967) and a marked improvement in the taste of the rapeseed meal has thus been achieved. As a result, the use of rapeseed meal in animal feeding has been considerably extended. During toasting, goitrogenic agents are inactivated probably due to the transformation of 5-vinyl-1,3-oxazolidin-2-thione to 5-vinyl-1,3-thiazolidinone (RUTKOWSKI et al., 1973). Unfortunately, extensive denaturation of protein, formation of Maillard-type compounds and incomplete removal of goitrogenic compounds preclude its use in human nutrition (RUTKOWSKI and KOZLOWSKA, 1969).

The successful production of soybean protein isolates and concentrates turned our attention to the use of water for the extraction of goitrogenic glucosinolates from rapeseed. The preparation of soya protein concentrates containing about 70 % protein, involves no serious difficulties (MARTINEZ, 1968; MEYER). Unfortunately, the relatively high starch and cellulose concentrations, even in dehulled rapeseed, accounts for the rather low protein content (50-60 %) in protein concentrates obtained from rapeseed meal (TAPE et al., 1970). Nonetheless it is important to emphasize that KOZLOWSKA and SOSULSKI (1972) and KOZLOWSKA et al. (1972) have managed to remove oxazolidinethione and to reduce the isothiocyanates level to about 0,5 mg/g seed mass, by three to five extractions with water.

If traces of glucosinolates are occluded in the protein, they can easily be removed by washing the isolate with water or polar organic solvents (KOZLOWSKA et al., 1964; RUTKOWSKI, 1972).
In developing a process for the isolation of rapeseed protein, we first established the main technological parameters of relevance. The results of these experiments are given below.

Material and methods

Commercial rapeseed of the winter variety was used. It contained 96 % dry mass, 41 % fat, 22 % protein (N x 6,25). Fat was removed from the ground seeds by extraction at 20° C with petroleum ether b.p. 40-60° C. In order to avoid denaturation of protein rapeseed meal was first dried at room temperature overnight and then in a vacuum drier under the pressure of 1 mm Hg at a temperature of 40° C, for 4 hours. The rapeseed meal obtained this way contained 0,2 % fat, 40,9 % protein and 16,7 % cellulose. Protein was extracted from the meal by means of acqueous hydrochloric acid and sodium hydroxide of concentrations up to 0,2 N. After removal of the insoluble residue by centrifugation, the protein was coagulated at pH 3,8 - 4,0 or pH 7,5 or by means of trichloroacetic acid.

The yield of rapeseed protein was assayed by determining by the Kjeldahl method.

The soluble rapeseed proteins were fractioned by gel filtration on Sephadex G150 using a column 3,2 x 50 cm, and 0,1 M disodium tetraborate for elution.

Results and discussion

Solvent volume. The volume of solvent regained after extraction of protein (V_1) is lower than the nominal volume used for the extraction (V) of the protein which is bound by the insoluble compounds, and this is proportional to the mass of meal treated (n). This relationship is described by the equation:

$$V_1 = V - A \cdot n$$

where: A ≅ specific solvent binding capacity of the insoluble part of the meal.

From the above equation it is possible to calculate the relative yield coefficient of the isolation process (K) as the quotient of actual (Y_p) and the theoretical (Y_t) yield from known conditions of extraction and coagulation:

$$K = \frac{Y_p}{Y_t} = \frac{V_1}{V} = \frac{V - A \cdot n}{V} = 1 - \frac{A \cdot n}{V}$$

For an experimentally determined value of A = 3,7 ml/g meal and the known values of V/n between 5 ml/g and 800 ml/g meal, the values relative yield coefficient are as below:

V/n (ml/g)	5	10	20	40	80	400	800
K	0,25	0,65	0,80	0,90	0,95	0,99	0,995

This indicates that for a single extraction, more than 20 ml of solvent per 1 g of meal gives yields of protein higher than 80 % of the theoretical yield that can be obtained under the given conditions of extraction and coagulation.

In further work we used 20 ml of solvent per 1 g of meal. POKORNY et al. (1964), studying the effect of extraction with 0,3 % NaOH, found the highest yield for V/n = 15 ml/g meal. But he recommended the use 10 ml/g meal for which be obtained the highest concentration of nitrogen in dry mass of extract. SOSULSKI and BAKAL (1969) used 20 ml/g of rapeseed meal, BERARDI et al. (1968) used 15 ml/g for the extraction of protein from cottonseed meal in a two-step process.

Generally, the values of V/n ratio used are in the range 10 ml/g to 20 ml/g for single or multiple step extraction processes. The choice of this ratio depends on economic considerations because on one the hand the higher solvent volumes increase the yield but concomitantly raise the costs of isolation and increase the consumption of water and energy.

pH of extraction. By changing the concentration of sodium hydroxide in the range from 0 to 2 mM/g of meal, and of hydrochloric acid from 0 to 4 mM/g of meal, the pH of extraction was changed while keeping the ratio of 20 ml of water per 1 g meal constant.

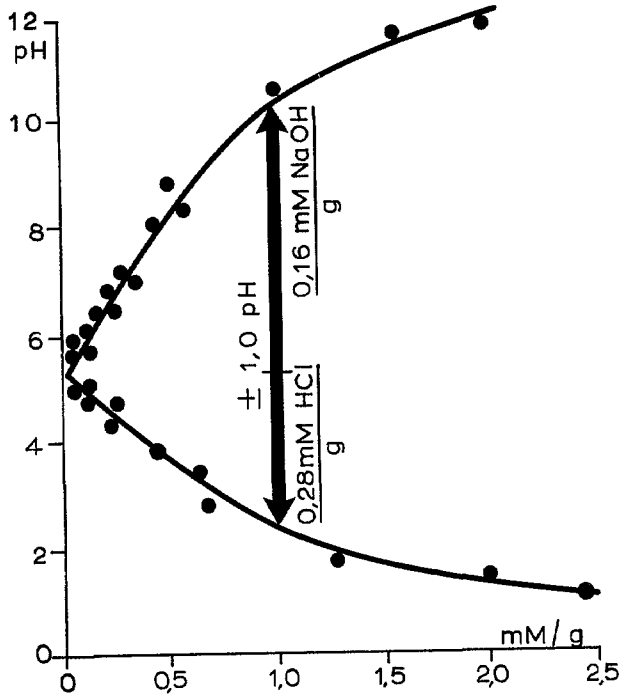
In the range of hydrochloric acid and sodium hydroxide concentration between 0 and 1 mM/g of meal, the pH of suspension changed in proportion to the dose of alkali or acid added (Fig. 1).

A change in pH of 1 unit in the range from 5,3 to 10,5, it was necessary to increase alkali on an average of 0,16 mM/g of meal, while in the range of 5,3 to 2,5 pH, 0,28 mM of acid per 1 g of meal was used. This indicates a higher buffer capacity of the meal in acid than in alkali solution. For the extraction of protein a pH from 1 to 3 and from 7 to 10 required 1 to 4 mM/g meal of hydrochloric acid or 0,4 to 0,75 mM/g meal sodium hydroxide.

In the pH range 3,8 to 0,8 and 6,5 to 9,6 the increase of solubility was approximately 15 % and the yield of protein about 11-12 % per 1 unit of pH change in acid or alkali solution. In the pH ranges 5,3 to 6,5, and 9,6 to 12, the changes of pH did not cause any major changes in solubility or yield of protein. The maximum solubility of the nitrigenous compounds of

rapeseed meal was about 77 % at pH 0,8 and about 86 % at pH 9,6.

Figure 1: Concentration of hydrochloric acid or sodium hydroxide and the pH value of rapeseed meal suspension

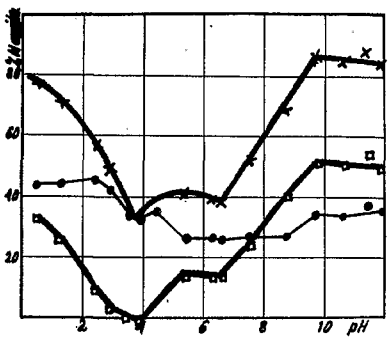


SOSULSKI and BAKAL (1969) obtained similar results (82-86 %) for different rapeseed varieties. The lowest level of nitrogenous compounds extracted was 34 % at pH 3,8 ± 0,2, and this confirms the previous results (KOROLCZUK and RUTKOWSKI, 1971). The maximum yield of protein isolate, about 52 % of total nitrogen, was obtained at pH 9,6 ± 0,2 and with the addition of sodium hydroxide at a level of 0,75 mM/g meal.

Nitrogen compounds that did not coagulate at pH 3,8 constituted 25 % to 40 % of the total nitrogen. Their level depended to some degree on the pH at extraction (Fig. 2). SOSULSKI and BAKAL (1969) had obtained similar results with different rapeseed varieties.

The shape of the curve for the solubility of nitrogenous components in the pH range 5 to 7 indicates the presence of a coagulating protein fraction in this pH range, which can be shown by changing the pH of a spernatant after removing the protein coagulating at pH 3,8.

Figure 2: Relationship between pH of rapeseed protein extraction and yield of non coagulating nitrogenous compounds at pH 3,8



total N-soluble N-non-coagulable
yield of protein

Time of extraction. The extraction of protein was carried out of room temperature and pH 7,1 to 7,3 or pH 9,6 to 9,7, under continuous stirring for 30 min. to hours.

It was found that the only important role played was that of the pH during extraction, while the time of extraction in the range examined had no effect (Fig. 3). Both, solubility of nitrogenous substances (S) and the yield of protein isolation were much higher for extraction at pH 9,6 than at pH 7,2. Because an increase in the time of extraction did not increase the yield, but even slightly decreased it, it was decided to use a 30 min. period of extraction in further experiments.

pH of coagulation. In order to establish the yield of protein, the parameters of coagulation of soluble rapeseed protein were established as follows: The rapeseed meal was mixed with 0,05 N sodium hydroxide, 20 ml per 1 g of meal, for 3 min. After centrifugation of the insoluble residue, the pH of the supernatant was lowered from pH 10 to 2,5 by adding 6 N hydrochlorid acid. From the intermediate stages the coagulated proteins were centrifugated and nitrogen was determined in the supernatants.

The yield of protein (Y) was calculated as the difference between the concentration of nitrogen after extraction (N_1) and after coagulation (N_2) according to the formula:

$$Y = \frac{V_1 \cdot (N_1 - N_2)}{n \cdot N_o} \cdot 100\ \%$$

Figure 3: Effect of pH and time of extraction on the solubility of nitrogenous components of rapeseed meal.
S = soluble, R = coagulate, NC = non-coagulate

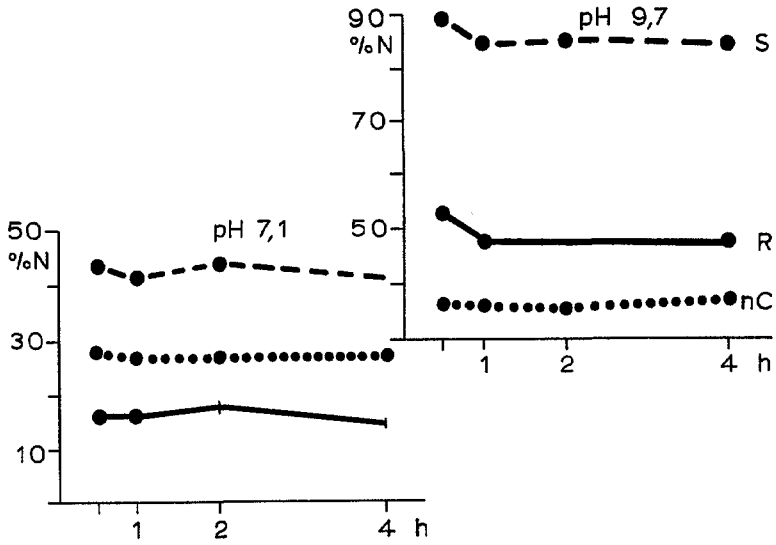
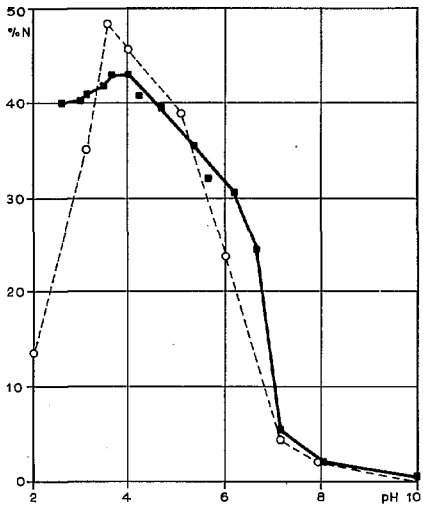


Figure 4: Yield of nitrogenous components at different pH

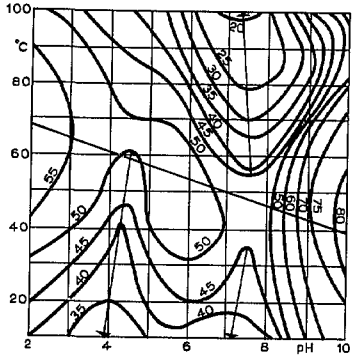


where: V_1 = the volume of solvent recovered after extraction,
n = the mass of meal used and
N_o = the concentration of nitrogen in the meal.

The highest yield of protein was obtained at pH 3,5 - 3,9 (Fig. 4). This agree well with the results of POKORNY et al. (1964) and FINLAYSON (1966) as well as with our earlier results (KOROLCZUK and RUTKOWSKI, 1971). Only SOSULSKI and BAKAL (1969) used pH 4,4 - 4,6 for coagulation of rapeseed protein.

Temperature during extraction and coagulation. The interdependence of the solubility of the nitrogenous components of rapeseed on temperature and pH has the character of a three-dimensional function (Fig. 5) as has

Figure 5: Extractability of nitrogenous components as a function of pH and temperature of extraction. The numbers over the contour lines represent the level of extractibility in per cent of total nitrogen of extracted meal.

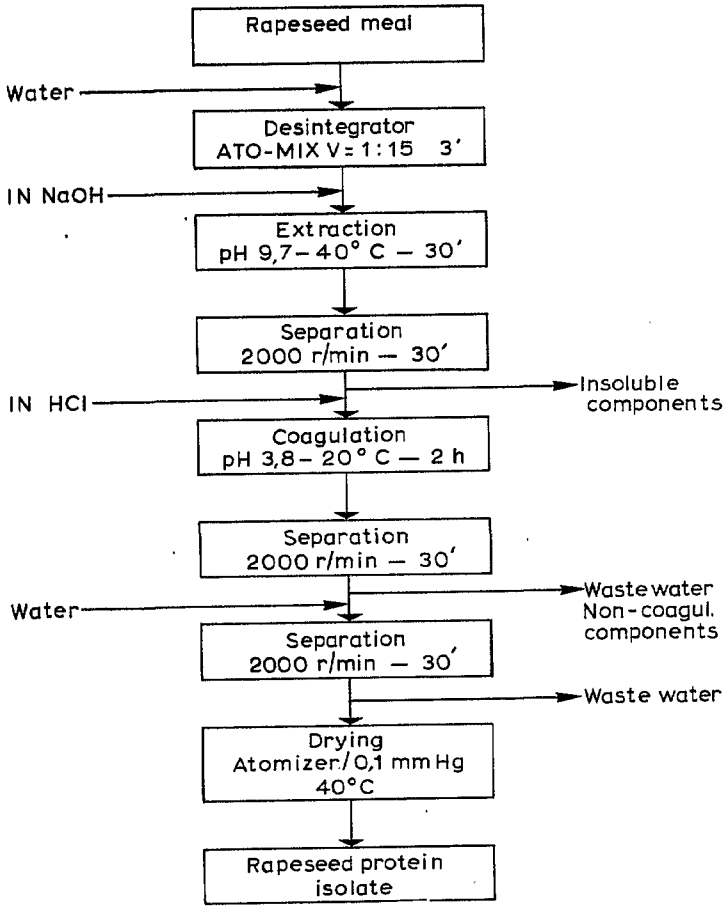


been discussed previously (KOROLCZUK and RUTKOWSKI, 1971). It has been reported that, as far as the alkali extraction is concerned, an increase in protein solubility occurs up to a temperature of 45° C, while the lowest solubility occurs at pH 3,8. This is of importance in establishing the conditions for protein coagulation obtained at the temperature of tap water.

Scheme of single step extraction. The experimental results led to the extraction scheme shown in Fig. 6. This type of extraction can be used both on a laboratory or a pilot plant scale.

Multi-stage extraction. Under industrial conditions, multi-stage extraction, which makes a more complete recovery of the protein from the raw material possible, is more important. We have shown the results of the

Figure 6: Scheme of single stage extraction of rapeseed protein



most characteristic examples of our experiments (table 1) into three-stage extraction in neutral (A) and basic (B) as well as two-stage basic (C) and acidic (D) media.

Nitrogen balance. The balance of nitrogen in two- and three-stage extractions and coagulation of protein from rapeseed meal is shown in Fig. 7.

For a three-stage extraction (A) of rapeseed meal at pH 5,5 - 5,7 - 6,1

Table 1: Two- and Three-Step Extraction of Rapeseed Protein

Step	Experiment							
	A		B		C		D	
	solvent	pH	solvent	pH	solvent	pH	solvent	pH
I	Water	5,5	Water	5,7	0,04N NaOH	9,7	0,1N HCl	1,9
II	Water	5,7	0,04N NaOH	9,7	Water	9,1	Water	1,9
III	Water	6,1	Water	8,7	-		-	

about 40 % of the total nitrogenous compounds were extracted from the meal. Only 17 % of nitrogenous material was recovered in the protein isolates and as much as 23 % nitrogenous substances constituted the non coagulating compounds.

For a three-stage extraction (B), where the first step was washing with water, the second alkali extraction and the third washing with water only about 10 % of total nitrogenous material was in soluble, while 50 % was the soluble protein.

When the two-stage alkali extraction method (C) was used, 44 % nitrogen was recovered in the form of an isolate of the protein coagulating at pH 3,8. A similarly high percentage (44 %) consisted of non coagulating nitrogenous compounds.

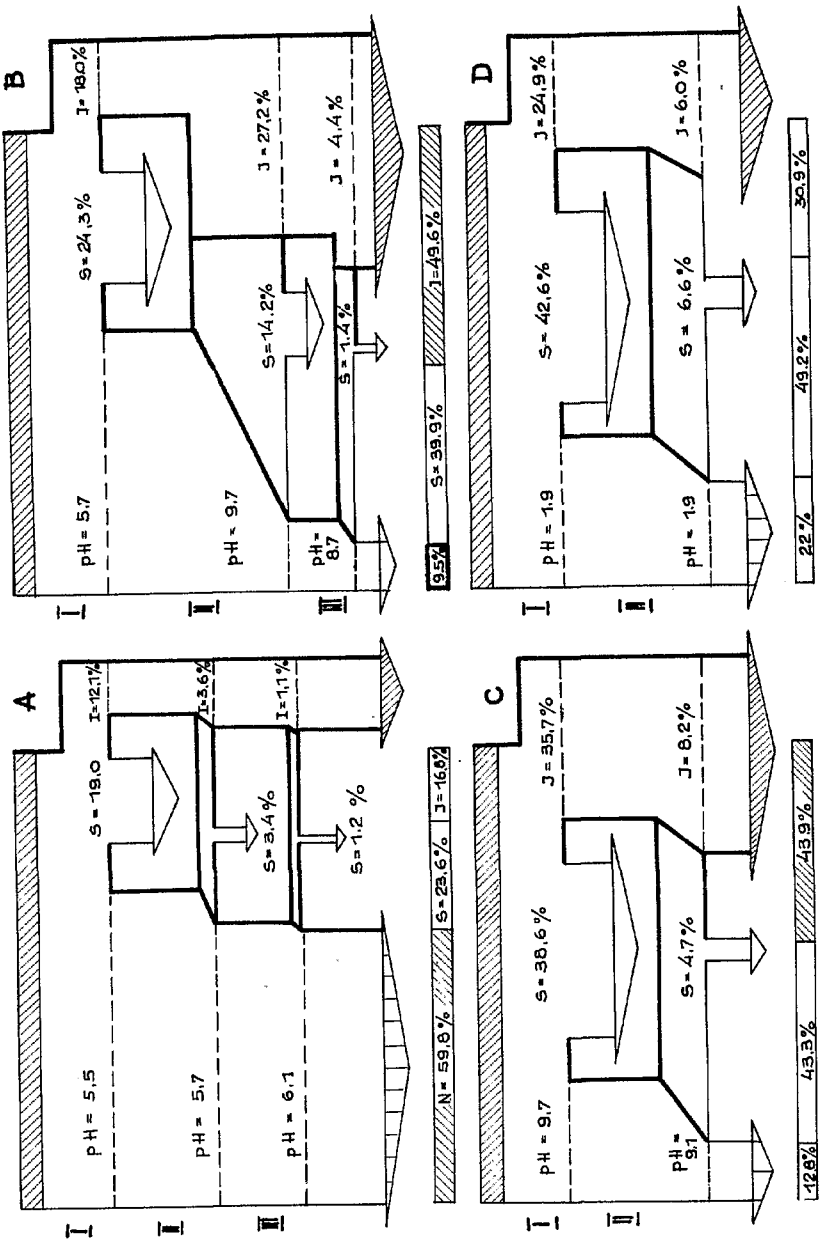
In acidic two-stage extraction (D), the yield of protein coagulating at pH 3,8 was only 31 % of total nitrogen while the level of non coagulated nitrogenous substances rose to 48 % of total nitrogen.

It can be concluded that the highest yield of protein can be obtained from rapeseed by a three-stage extraction following the scheme: water - sodium hydroxide - water.

A comparison of the two-stage extraction with the three-stage extraction shows that two-stage extraction may be satisfactory, although it is 5 -10% less effective, because it is much simpler. It seems useless to employ the water extraction method, despite the fact that an isolate of low ash content can be obtained.

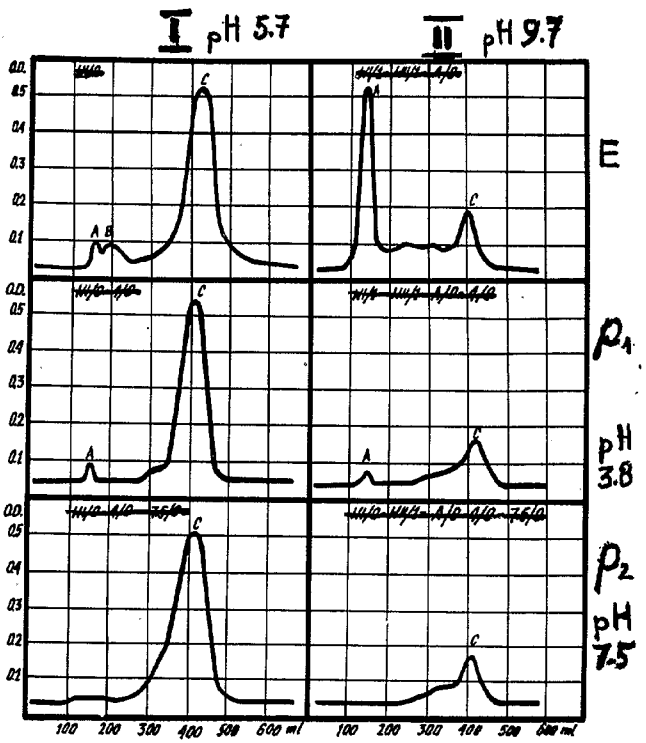
The results reported agree with the data of other authors (POKORNY et al., 1964; SOSULSKI and BAKAL, 1969) who used a five-stage extraction with 0,2 % NaOH.

Figure 7: Scheme of two-stage (C, D) and three-stage (A, B) extraction of rapeseed protein



Gel filtration. A study of the nitrogenous substances obtained during a three-stage water-alkali-water extraction (Fig. 8) has shown that two protein fractions occurred in the water solution after the first stage of extraction at pH 5, 7. The molecular weights of these were in the range of 100 000. The low molecular substances (peak C) had molecular weights of 100-1000.

Figure 8: Separation by gel filtration of non-coagulable nitrogenous components obtained by three-stage extraction of rapeseed meal I and II = stage of extraction; E = extract; P_1 = precipitate at pH 3, 8; P_2 = precipitate at pH 7, 5.



After the second-stage the alkali extraction, the main component of the solution was a protein fraction (A) having a molecular weight of about 100 000. After coagulation of the protein at pH 3, 8, only traces of high molecular compounds (A) were found in both, water and alkali extracts, and the initial level of low molecular substances (C) remained unchanged.

Additional coagulation at pH 7, 5 resulted in all proteins being coagulated and in the supernatant only the low molecular components (C) remained at the initial level.

Color of isolates. The protein isolates obtained from rapeseed meal by alkali extraction were characterized by a slight brown color. Attempts to remove the pigment from the isolate by repeatedly dissolving the protein in alkali solution and coagulation at pH 3,8 failed to give positive results. It even caused further darkening of the protein isolate. The brown pigment of the isolates was soluble in neither polar and nonpolar organic solvents and it could not even be removed by gel filtration.

More satisfactory results were obtained by using water for the initial extraction of the meal. Additional washing of the isolates with polar organic solvents such as 50 % methanol, ethanol, isopropanol, acetone etc. led to the removal of the traces of flavones and counteracted the browning of the protein isolates during drying.

Initial extraction of the meal with water and additional purification of the isolates by means of alcohol, led to dark cream isolates. This treatment also ensured the removal of traces of isothiocyanates which would otherwise have remained in the protein isolate.

Conclusion

Rapeseed constitutes a good raw material for the isolation of vegetable protein. Extraction is best done using water followed by 0,04 N NaOH followed by water. Other parameters are as follows: temperature 40°C ± 5, time below 30 min., ratio of solvent to meal 20 ml/g. Conditions for the coagulation of protein are pH 3,8 ± 0,2, and temperature 20°C ± 5. The yield of protein accounts for 50 % total nitrogen.

References

1. BERARDI, L. C., W. H. MARTINEZ and C. J. FERNANDEZ (1968): 28th Annual Meeting Inst. Food Technol. 23, 5

2. FINLAYSON, A. J. (1966): Can. J. Biochem. 44, 997-1004

3. KOROLCZUK, J. and A. RUTKOWSKI (1971): J. Am. Oil Chem. Soc. 48, 398-399

4. KOZLOWSKA, H., J. CHUDY, A. JAKUBOWSKI and K. MODZELEWSKA (1964): Tl. Sr. Pior 8, 128-139

5. KOZLOWSKA, H. and F. W. SOSULSKI (1972): Paper 73, 46th Annual Meeting of the Am. Oil Chem. Soc. Ottawa, 24-28. Sept. 1972

6. KOZLOWSKA, H., F. W. SOSULSKI and C. G. YOUNGS (1972): Can. Inst. Food Sci. Technol. J. 5, 149-154

7. LANZANI, A., J. KOROLCZUK, A. RUTKOWSKI and G. JACINI (1972): Riv. Ital. Sost. Grasse 49, 549-562

8. MARTINEZ, W. H. (1968): Conference on Protein-rich Food Prod. from Oilseeds, New Orleans, 15.-16. May, 1968 p. 33-39

9. MEYER, E. W.: Ibid, p. 95-100

10. POKORNY, J., Z. SEFR and M. VODNICKA (1964): Prumysl Potravin 15, 285-286

11. RUTKOWSKI, A. and H. KOZLOWSKA (1967): Oléagineux 22, 173-175

12. RUTKOWSKI, A. and H. KOZLOWSKA (1969): Oléagineux 24, 687-690

13. RUTKOWSKI, A. (1972): Riv. Ital. Sost. Grasse 49, 416-427

14. RUTKOWSKI, A., H. KOZLOWSKA and J. CWIK (1973): Riv. Ital. Sost. Grasse 50, 10-20

15. SOSULSKI, F. W. and A. BAKAL (1969): Can. Inst. Food Sci. Technol. J. 2, 28-32

16. TAPE, N. W., Z. I. SABRY and K. E. EAPEN (1970): Can. Inst. Food Sci. Technol. J. 3, 78-81