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ISOLATION AND FUNCTIONAL PROPERTIES OF

PROTEIN FRACTIONS FROM RAPESEED FLOUR

(Brassica campestris L. var. Echo)

By

LAKSHMAN PUNNIYADASA KODAGODA

B.Sc. University of Ceylon, 1961

M.Sc. University of Tokyo, 1965

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ABSTRACT

A three stage method of extraction of proteins from rapeseed flour sequentially with water, 0.1 N hydrochloric acid, and 0.02 N sodium hydroxide has been developed. The first water extract contained mainly acidic and neutral proteins whereas the second hydrochloric acid extract contained basic proteins, which migrate to the cathode by pH 8.8 gel electrophoresis. This can partially be explained by higher arginine content.

Ash content in the second hydrochloric acid extract, approximately 36%, was reduced to less than 10% by treating with 0.05 M oxalic acid.

The water extract may be a good supplement to other vegetable products because of high lysine content.

The approximate yields of proteins in the preparation of isolates were 11, 7, 41 and 61% respectively for the water, hydrochloric acid and sodium hydroxide extractions and for the single stage sodium hydroxide extraction. Proteins were lost in the whey during isoelectric precipitation, with a concomitent loss of a considerable amount of cystine and methionine.

The emulsifying capacity of the water extract was better than that of the other extracts: 45 and 35 ml of corn oil per 100 mg of protein isolate respectively. The concentrates showed a similar trend, but the volume of oil

was approximately 25% less for all preparations.

In baking studies, the replacement of 5% of the wheat flour with rapeseed protein isolates prepared by iso-electric precipitation caused an approximate 10 to 15% decrease in loaf volume. The bread prepared with the concentrates decreased loaf volume by about 20%.

The loaf volume was restored in most cases by adding 0.5% Atmul 124 (a mono- and diglyceride mixture manufactured by Atlas Chemical Industries) to the dough. With the isolates from the first water and second hydrochloric acid extracts an increase in loaf volume of 10 to 15% was observed over the control. With the isolates from the third sodium hydroxide and single stage sodium hydroxide extractions the loaf volume was restored without any increase.

The bread prepared from the concentrates together with emulsifier showed a decrease of 12% in loaf volume except the third sodium hydroxide extract which had a 6% increase.

Whip tests showed the best expansion of meringue when the protein isolate from the hydrochloric acid extraction was used at a level of 3% replacing egg white protein, an even better result than whipping the egg white alone. All other isolates showed a decrease in expansion. The concentrates except the water extract showed a slight decrease.

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The definition for rapeseed protein concentrates and isolates as products extracted once from flour and those which were processed one step further, respectively, was used.

It is generally accepted for soyprotein products that soyprotein isolates and concentrates are defined as products containing a minimum of 70 and 90% protein (N x 6.25) on a moisture free basis, respectively. However, rapeseed flour used for extraction of protein in this study was heat-treated to decrease glucosinolates before extraction, which made residual fat extraction difficult. With an intention of practical application, no drastic solvent extraction for removing these residual lipids were attempted.

The protein content of the concentrates and isolates on a moisture and fat free basis (N x 6.25) are tabulated below:

	Concentrates[1]	Isolates[1]
First water extraction	64.6	86.8
Second hydrochloric acid	45.8	86.6
Third sodium hydroxide	75.6	87.5
Single stage NaOH extraction	76.0	91.3

1. These results are very close to the value obtained for soybean products.

PART I

ISOLATION OF PROTEIN FRACTIONS FROM RAPESEED FLOUR

(Brassica campestris L. var. Echo)

CHAPTER I

INTRODUCTION

Rapeseed (Brassica sp) is produced in large quanti-
ties in Canada especially for its oil used in the manufacture
of edible products such as margarine, shortenings and edible
oils. Fifty percent of the oil seed crop in Canada is
rapeseed (29). With the large tonnage of the oil seed crop
produced annually for the oil, the resulting meal has become
an important source of meal protein. Compared to the meal
produced previously, the present day meals are of a better
quality due to improved techniques used in the processing of
the meal and flour.

The glucosinolates which are the precursors of
toxic principles and the high fiber content of rapeseed flour
and meal are the two major limiting factors against the
utilization of them as a protein supplement in human foods.
The colour of the isolates and concentrates may also be
another obstacle for the utilization of them in supplementary
foods. Smith (84) indicated that the yield of isolated
proteins from sunflower and flux meal were low for commercial
production. The yield obtained from rapeseed by Owen and
Chichester (65) using their extraction procedure was 18%.
Sosulski and Bakal (86) had a protein recovery of 25%. Thus
it is noted that there is a loss of protein from rapeseed
isolated by isoelectric precipitation.

Most of the recent work on rapeseed protein has been carried out at the National Research Council, Prairie Regional Laboratory, Saskatoon. Finlayson et al. (37) have been conducting extensive work on physical and chemical properties of rapeseed protein fractions purified by various chromatographic techniques. Bhatty et al. (14) have isolated different protein fractions from rapeseed protein soluble in salt solutions by using column chromatography.

Rapeseed (Brassica napus L. var. Nugget) contained at least 9 chromatographically distinguishable protein components. Of these, there are 2 major proteins (12 S and 1.7 S), which together contained 35% of the seed protein and the 12 S protein is an aggregate of several protein chains and has a large molecular weight (14). These workers have shown that the 12 S protein fraction was chromatographically and electrophoretically homogeneous in salt solutions between pH 8.0 and 9.5, but dissociated into subfractions at pH values below 3.5 and in urea solutions.

In this study research was initiated to find an economical source of vegetable protein isolates for the supplementation of protein deficient diets. The primary objective was to develop an extraction procedure to obtain from rapeseed flour nearly colorless protein isolates with different characteristics which had some functional properties suitable for food processing. The proteins

resulting from this study were compared with that of casein and soybean protein isolates and concentrates in order to evaluate their importance.

CHAPTER II

LITERATURE REVIEW

Origin of Brassica napus L. and Brassica campestris
L. is not well documented. The earliest direct reference to
the oilseed rapes is found in the ancient Sanskrit writings
of 200 to 1500 B.C. (82). Sinskaia (83) after a study of
form found within this species in Europe and Asia, suggested
that the center of origin of both turnip and turnip rape
would ultimately be located in Asia. On the other hand
Sinskaia (83) noted that all cultivated Asian turnip rape is
of the summer form, and thus concluded that winter turnip
rape must have originated under maritime climate such as the
Mediterranean. It is possible that the Indian and European
varieties were separated at an early stage in the develop-
ment of the species and evolved along different lines.

In western Canada rapeseed was first grown commer-
cially in 1942, a war measure to supply oil for lubrication
of marine engines. A major by-product of this oil extrac-
tion is the meal. With the present use of prepress -
solvent extraction which is not subject to high temperatures
during processing, a meal comparable to soybean is produced.

Rapeseed is a crop well adapted to Canadian
conditions. The production has increased rapidly until
supply of the seed has gradually exceeded domestic demand.
As a consequence in recent years Canada has exported more
rapeseed than all other countries combined.

Accurate statistics of trade in rapeseed meal are
not readily available. However, it has been established
that the major producing countries had an annual export of
approximately 30,000 metric tons of meal in the period 1958
to 1962, (22). In the year 1968/69 the production had
increased to 5,323,000 tons and for the year 1969/70 there
was a slight decrease with 5,250,000 tons of rapeseed (23).
Of this approximately 75% is produced and consumed in Asia.

In Canada 2 types of rapeseed are produced:
Brassica napus L. (Argentine rape) and Brassica campestris L.
(Polish rape). Of the 2 species the Argentine variety has
a greater potential for seed and oil than the Polish rape.
But the varieties of the latter are preferred, in Canada,
because they mature 10 to 14 days earlier (30). Thus summer
forms are grown, as even the most hardy turnip rape
varieties will not consistently survive on the open plains
of western Canada. The turnip rape varieties Echo and Arlo
occupy 70 to 80% of the rapeseed acreage of western Canada.
Rapeseed ranks fifth in world production among vegetable
oils, exceeded by soybean, peanut, cotton seed and sunflower.
Canada which is the largest exporter of rapeseed has 50% of
the oil seed crop (29).

The large amount of meal enables a substantial
quantity of protein to be available for consumption. The
rapeseed crushed in modern mills yields approximately 40%
oil and 50% oil meal and the remainder is moisture. The

protein rich meal or flour, having about 40% crude protein, is presently utilized for livestock and poultry feeding.

The meal currently produced differ from those that were available previously. Modern meals are subjected to less heat during processing and the amount of oil left in the meal is greatly reduced, compared to expeller meal, in which damage to the protein had occurred during processing. Because of the reduction in the amount of heat used in processing the meals are of much better quality than those produced years ago.

Alexander and Hill (1) found that autoclaving at 15 lb pressure and dry heating at 121°C sunflower oil meal prepared by solvent extraction caused a marked destruction of lysine in the meal. These treatments however did not affect methionine. Morrison et al. (59 and 60) observed that the nutritional value of sunflower oil meal increased as the processing temperature was increased. However, Clandinin and Robblee (19) attributed the poor quality of the protein from sunflower oil meal to excessive processing temperature.

It has been reported by Craig (24) that the growing conditions of rapeseed affect the composition of seed constituents. For instance the degree of unsaturation of fatty acids was related to the environmental conditions during growth. The glucosinolate (formerly known as thioglucoside) content of the rapeseed is proportional to

the available sulfur in the soil. This has been demonstrated
by Wetter (99) and Josefsson and Appelqvist (50).

Earlier it was reported that plants of Brassica
napus L. var. nugget grown in soils deficient in sulfur
produced seeds with a lower content of certain protein
fractions and resulted in different chromatographic behaviour
of the 12 S globulin than seeds produced from adequate
fertilized plants (37). These findings suggest that the
availability of soil sulfur has a significant effect on
protein synthesis.

The composition of rapeseed is highly inconsis-
tent. Thus there are many compositional differences from
species to species, variety to variety and even within a
variety. This is mainly due to the highly self-fertile
nature of Argentine rape and under field conditions they
are largely self-pollinated (62).

Bell (13) indicated in a review that rapeseed
meal when incorporated into poultry rations at levels of
10% or higher was goitrogenic and detrimental to growth.
In a report published by Clandinin et al. (20) it was
proved that Argentine rape (Brassica napus L.) was more
detrimental to growth of chicks and goitrogenic than the
meal originating from Polish rape (Brassica campestris L.).
Ten percent Argentine rape meal in the ration depressed
chick growth, whereas much as 15% Polish rapeseed meal in
the ration did not result in appreciable growth depressions.

Five percent Argentine rapeseed meal was shown to double the thyroid to body weight ratios while 15% Polish rapeseed meal was required to produce a similar effect, on chicks. Similar results were obtained by Renner et al. (75).

Limitations of using rapeseed is due to the presence of low molecular weight sulfur compounds in the seeds, some of which may be released through enzyme action causing metabolic disturbance.

Clandinin et al. (20) reported that isothiocyanate content was markedly affected by variety and no consistent effect of location was apparent. The thiooxazolidine content on the other hand was not only affected by variety, but also appeared to be influenced by the environmental conditions.

The three major glucosinolates of rapeseed meal are gluconapin, glucobrassicanapin and progoitrin. In gluconapin and progoitrin the radical R is $CH_2=CH-CH_2-CH_2$ and $CH_2=CH-CH(OH)CH_2$ respectively (5). On enzyme hydrolysis these glucosinolates produce 3-butanyl isothiocyanate, 4-pentyl isothiocyanate and 5-vinyl oxazolidine thione, respectively. Sandburg and Holly (77) indicated that this system is made up of 2 entities, namely thioglucosidase and sulfatase. It was suggested by Pitt-River (68) that the main mustard oil of rapeseed, 3-butenyl isothiocyanate might be converted to 5-vinyl thiooxazolidone by oxidation.

Gmelin and Virtanen (43) suggested that the goitrogenic effect of thiooxazolidones is due to the fact

that they inhibit the synthesis of thyroid hormones. This effect cannot be overcome by high doses of iodine.

Another type of goitrogenic substance belonging to the Brassica family has a primary influence on the uptake of iodine by the thyroid gland. This influence can be prevented by increasing the amount of iodine dose (43). Literature indicates that salts of thiocyanate (SCN^-) represents this type of crucifers.

It has been proved by Ettlinger and Lundeen (36) analytically that sinigrin is the potassium salt which rearranges during enzyme cleavage to allyl isothiocyanate.

Feeding experiments have shown that intact thioglucosides are not particularly harmful but rather toxic due to their hydrolytic products such as isothiocyanates, oxazolidinethiones and nitriles which are liberated by myrosinase in the seed in the presence of moisture. The oxazolidinethiones are mainly responsible for the enlargement of the thyroid (18). Oxazolidinethiones also appear to be the compound primarily, if not entirely, responsible for goitrogenicity. However it has been shown that isothiocyanates can cyclize to form oxazolidinethiones, which may account for the rather similar effects of the 2 types of rapeseed compounds.

A survey of the Canadian rapeseed cultivers (Brassica napus L. and Brassica campestris L.) indicates a significance within species cultivar difference in glucosinolate content (100). However differences between cultivars were relatively small and none of the cultivars was found to

be free or practically free of glucosinolates. Cultivars from Japan and India were found to be free of 4-pentyl isothiocyanate and or 5-vinyl oxazolidine-2-thione.

In present day processing techniques, the enzyme is destroyed in the initial processing by steam heat and the goitrogenic factors are left bound in the original glucoside complex (101).

Eapan et al. (32) have developed a wet method for inactivating the myrosinase, the enzyme responsible for the production of toxic isothiocyanates and oxazolidinethiones from the substrate glucosinolate. They were also able to remove glucosinolates by aqueous extraction (33) by this process the fibrous hulls by air clarification to obtain a hull free, creamy coloured flour and a gray meal. Ballester et al. (9) developed a water washing technique for meal detoxification that was effective in eliminating glucosinolates.

The safest and the most economical solution however is either to breed strains with little or no sulfur containing glucosides in the seeds, or to select lines which produce only harmless isothiocyanates upon glucosidic hydrolysis. It is shown that sulfur fertilization has marked effect on the total content of these compounds in the seeds, but regardless of the level of sulfur applied, some varieties are consistently low in total isothiocyanates.

Until 1960 all varieties of rapeseed produced oil containing 20 to 25% erucic acid, a monounsaturated 22 carbon chain fatty acid. Stefansson and Hougen (87) isolated rapeseed

strains which produced oil practically free of erucic acid.
Oleic acid replaced erucic acid as the major constituent of
these oils. Species containing oleic acid at levels higher
than 70% have been obtained. Beare et al. (11) showed the
deficiency of saturated fatty acids would result in undesirable
effects of the oil and particularly the residual oil in the
meal when used as feed, or for the preparation of isolates
or concentrates.

It has been reported by Sosulski and Bakal (86)
that acid precipitation of alkali extracts of protein from
rapeseed gave low yields with brownish color. The protein
isolates obtained from sunflower was dark green in color due
to the formation of an insoluble complex between chlorogenic
acid and protein (84). There is no evidence that chlorogenic
acid occurs in rapeseed. The development of a green color is
believed to be due to oxidation of chlorogenic acid, a tannin
like compound present in appreciable amounts in sunflower seeds
was explained by Malic et al. (56).

Zdenek et al. (104) have used countercurrent
extraction for protein from rapeseed meal. In comparison with
batch extraction under similar conditions, extracts obtained
by countercurrent extraction with sodium hydroxide or sodium
bicarbonate contained more dry matter, total nitrogen and
precipitatable protein. This procedure is not economically
feasible due to the complexity of the operation.

Gheyasuddin et al. (41) obtained almost colorless

sunflower protein isolates by maintaining the pH at 10.5 during extraction with 0.25% sodium sulfite. The precipitated proteins were washed with 50% aqueous isopropyl alcohol, probably able to break H-bonds, to produce the nearly white isolates. It is known that polyphenols at acid pH remain combined with proteins by unusually strong H-bonds as shown by Lommis and Battaile (54). Stronger H-bond breaking compounds, such as 50% aqueous ethanol, will be even more effective in preventing the interaction between polyphenols and proteins. However such compounds denature proteins by destroying their native structure.

Goding et al. (44) has also reported purification of 12 S globulin isolated from each of two species of rapeseed (Brassica napus L. and Brassica campestris L.). They have been shown to be similar in terms of amino acid composition, amino terminal amino acid, number of subfractions and the carbohydrate content.

While the present work was in progress, Owen and Chichester (65) reported a process for the production of nontoxic rapeseed protein isolates and an acceptable feed by-product. They extracted the rapeseed presscake with sodium chloride solution, precipitated with acid and obtained an isolate with light tan color after repeated washing with water.

Tape et al. (90) developed an aqueous extraction procedure for the removal of thioglucosides from crushed

rapeseed to produce a flour suitable for human consumption.
The defatted rapeseed flour and meal produced by standard
oil extraction and air clarification techniques contain over
50% and 30% protein respectively. The thioglucoside content
of each fraction was almost free.

Lo and Hill (53) have prepared protein concentrates
from rapeseed meal by extracting with 10% sodium chloride
solution followed by filtration to remove the seed coats and
dialysis against running water and freeze dry the desalted
rapeseed milk. The method yields up to 75% of the original
meal nitrogen in the form of products containing 61 to 76%
protein (N X 6.25). The product showed a considerably greater
content of ash and lower content of crude fiber and gluco-
sinolate than did the starting material. The amino acid
composition was similar and the phosphorus level did not
change during processing.

Janson (49) has reported columns for large scale
gel filtration on porous gels for the fractionation of
rapeseed proteins and insulin. From this method he obtained
a broad peak containing at least 4 main components. All of
these have been basic proteins having I_p greater than 10 and
with molecular weights approximately 15 to 20,000.

Korolezuk and Ruttowski (52) studied the extraction
of nitrogenous compounds of rapeseed meal in relation to pH
and temperature. They obtained high extractability of
nitrogenous compounds more than 80% of total nitrogen at pH

9.5 to 10 and 30 to 45°C. For the region of pH 2 temperatures around 60°C gave about 55% extractability.

CHAPTER III

EXPERIMENTAL MATERIALS AND PROCEDURES

1. Materials

The rapeseed flour (Brassica campestris L. var. Echo) was a generous gift from the Food Research Institute of the Canada Department of Agriculture, Ottawa. The flour was processed by a procedure developed by Tape et al. (90) so as to free the product of toxic thioglucosides and hulls.

Residual oil in the flour was removed by extracting with petroleum ether (57). For the preparation of proteins for electrophoresis the flour was extracted with chloroform:methanol (2:1 v/v) mixture to improve the resolution.

All chemicals were analytical grade obtained from Fisher Scientific Co. Ltd., Vancouver, B.C. Ampholine pH 3 to 10 was obtained from Pharmacia Uppsala, Sweden.

,2. Procedures

A. Determination of nitrogen ,

Semi micro Kjeldahl procedure using an Aminco dis-
tillation apparatus (see Plate 1) was used for the determina-
tion of total nitrogen. The materials were digested in the
presence of potassium sulfate:selenium powder (100:1 w/w)
mixture together with a piece of copper wire with concentra-
ted sulfuric acid. Fifty ml of the distillate was collected
in 3% boric acid containing a few drops of mixed indicator,
by steam distillation with 40% sodium hydroxide. Protein was
calculated by multiplying the nitrogen content by the constant
5.7.

B. Determination of moisture and ash

Moisture and ash were determined according to the
standard A.O.A.C. 1970 procedure (6).

C. Determination of lipids

Lipids were extracted from the isolates and concen-
trates by wetting with 1.0 ml of alcohol and then 10.0 ml
hydrochloric acid (24:11 by volume) was added and hydrolyzed
for 40 min. at 80°C in a water bath. After hydrolysis 10.0
ml alcohol was added and the mixture was cooled. These were
then transferred to Mojonnier G 3 Fat Extraction Flasks. The
container was rinsed with 20.0 ml of ether added in 3 equal
portions. The flasks were stoppered and shaken vigorously
for 1.0 min., then 20.0 ml petroleum ether (b.p. 30-60°C)
added and shaken again vigorously for 1.0 min. The ether

PLATE I

AMINCO DISTILLATION APPARATUS FOR NITROGEN

DETERMINATION
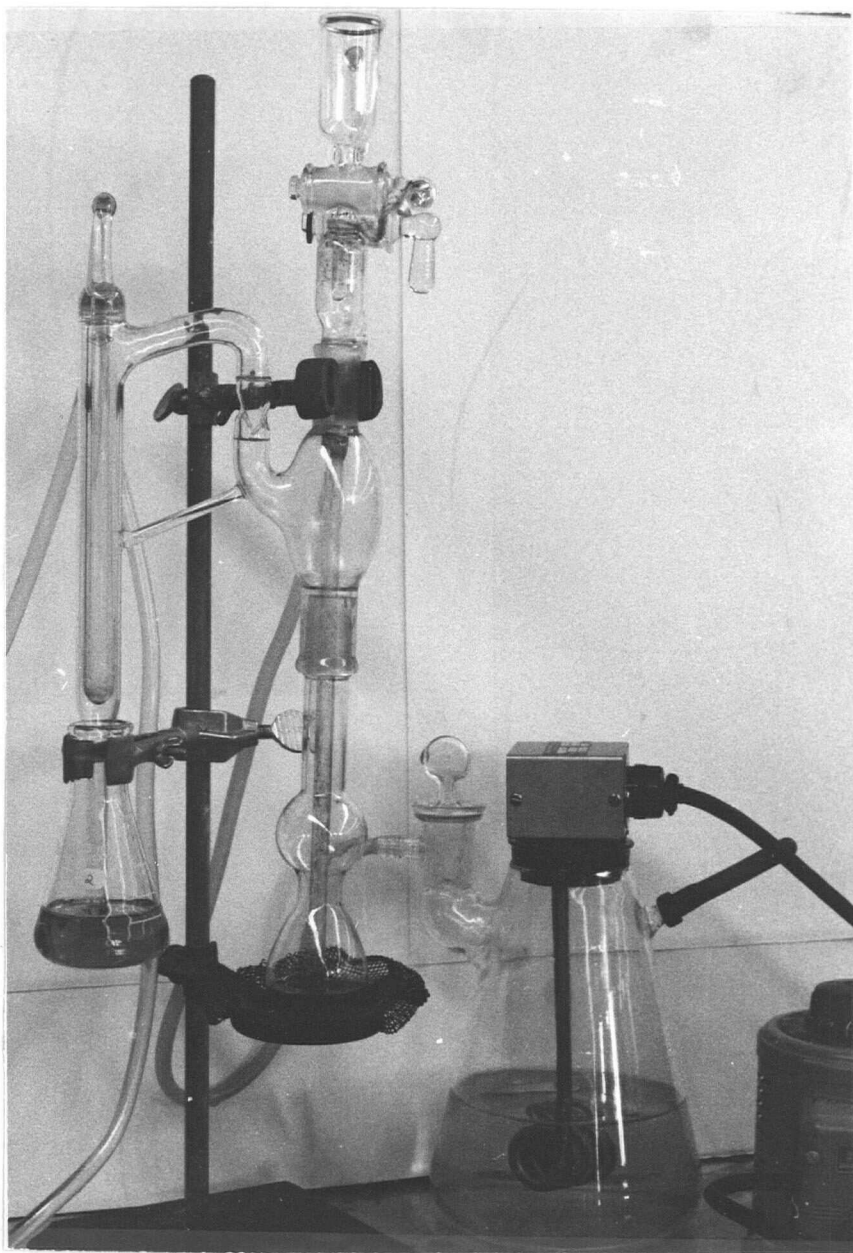
layer was allowed to separate from the aqueous phase. The other layer was then drawn off as much as possible and filtered through a cotton wool packed stem of funnel to let ether pass freely into weighed beakers containing broken glass.

Reextracted the remaining aqueous phase in the tube twice with 15.0 ml each of ether as described before. Draw the ether layer containing the fat into the same beaker. Wash the funnel and the tip of the funnel with a few ml of equal volumes of ether and petroleum ether. The ether was slowly evaporated on a steam bath inside a fume hood. The beakers containing the fat were dried in an oven at 100°C for 90 min. These were then allowed to stand in air and weighed to constant weight. The results are expressed as % fat by acid hydrolysis.

D. Determination of phosphorus

Phosphorus content of the isolates and concentrates was estimated by the method of Fisk and Subbarow (38). The organic matter was destroyed by the wet oxidation and the formation of phosphomolybdic acid complex which is quantitatively reduced to heteropholyblue color, was measured by spectrophotometry.

Samples of the isolates and concentrates were digested with 6.0 ml of nitric acid: sulfuric acid: perchloric acid (5:1:2 by volume) mixture until white acid fumes evolved for 2 to 3 hrs. On cooling the digest was trans-

ferred to a volumetric flask with 0.1 N hydrochloric acid.

To aliquots of the digest was added 5.0 ml of 2.5%
ammonium molybdate in 5 N sulfuric acid and diluted to 45 ml
in a 50 ml volumetric flask. To this was added 2.0 ml of
15.4% 1-amino 2-naphthol 4-sulfonic acid : sulfite-bisulfite
dry mixture in warm water. Made up to volume and the contents
well mixed, the absorbance measured at 680 nm after 25 mins.
in a Bausch and Lomb Spectronic 20 Colorimeter/Spectro-
photometer. Concentration of phosphorus was read from a
standard curve prepared with sodium phosphate.

E. Determination of Calcium

Calcium was determined by the method described by
Ntailianas and Whitney (61). The samples of the isolates
and concentrates were ashed at 400°C for 2 hrs. in a muffle
furnace. The ash was dissolved in 0.1 N hydrochloric acid
and made to volume. The aliquots of the extract were added
5.0 ml of 0.024 M disodium dihydrogen ethylenediamine tetra-
acetate dihydride and 8 N potassium hydroxide to increase
pH to 13 followed by 3 drops of 0.2% calcine (aminomethyl-
fluroscein) indicator in dilute sodium hydroxide. The color
of the sample becomes pink. Back titrate with standard
0.024 M calcium chloride solution, prepared by dissolving
calcium carbonate in minimum amount of hydrochloric acid
and diluting to volume. The end point is marked by a color
change in solution to a permanent green color. The titra-
tion was carried out against a black background.

The amount of calcium was calculated after making
corrections for the blanks. Results are expressed as
percentages.

F. Determination of total sulfur

Total sulfur was determined in the isolates and
concentrates of rapeseed proteins by the method of Bardsley
and Lancaster (10). The samples were mixed with 0.5 g
sodium bicarbonate thoroughly and an additional 0.5 g bi-
carbonate was laid over and was fused at 600°C for 4 hrs.
The fused material was then extracted with 25 ml of 0.46%
sodium dihydrogen phosphate in 2 N acetic acid in 3 portions.
After the reaction subsides, allow to stand for some time
and filter through a Whatman No. 1 filter paper.

To 10.0 ml aliquots add 1.0 ml of 0.5% freshly
prepared gum acassia in 50% acetic acid, and 1.0 ml 6 N
hydrochloric acid, and 0.5 g finely ground barium chloride.
After one min. contents were shaken to dissolve the barium
chloride. The absorbance was read after 5 mins. at 420 nm
in a Spectronic 20 spectrophotometer. The amount of sulfur
was read off from a standard curve prepared with magnesium
sulfate.

G. Determination of Carbohydrates

Carbohydrates were determined in the rapeseed pro-
tein isolates and concentrates by a method developed by Dubois
et al. (31) with a few modifications. Samples were mixed
with 1.0 ml of 5% aqueous phenol (w/w) followed by 5.0 ml

concentrated sulfuric acid added directly with a wide opening
pipet. After standing for 10 mins. the contents were mixed
and the absorbance was read at 480 nm on a Spectronic 20
spectrophotometer. The concentration of carbohydrate was
read off from a standard curve.

H. Determination of crude fiber

Crude fiber determination is based on the material
not soluble in acid and alkali, which is probably due largely
to cellulose together with lignin and pentosans. The amount
of crude fiber is roughly that portion of food which is not
appreciably digestible by most monogastric animals. Crude
fiber is estimated from the loss on ignition of dried
residue remaining after digestion of material with 0.255 N
sulfuric acid and 0.313 N osdium hydroxide under specific
conditions.

Samples of the rapeseed isolates and concentrates
were mixed with 0.5 g predigested asbestos and 100 ml of
0.255 N sulfuric acid added and boiled for 30 mins. in
digesting apparatus, adjusted to boil in 15 mins. together
with a few drops of antiform A emulsion diluted with water
and containing some boiling chips. Filter the digest
through a California polyethylene buchner funnel fitted
with a 200 mesh screen, with suction. The beaker is rinsed
with 35 ml boiling water and washed, the residue with 3
portions of 25 ml boiling water. The residue is then trans-

ferred back to the beaker and boiled for 30 mins. with 100 ml

boiling 0.313 N sodium hydroxide as described before. The

beaker is rinsed with 35 ml boiling water and the residue is

washed with 15 ml of boiling 0.255 N sulfuric acid and then

with 3 portions of 25 ml boiling water followed by 15 ml of

alcohol.

The residue is removed from the funnel by tapping

into an ashing dish and heated for 2 hrs. at 130°C. It is

then cooled in a desiccator and weighed. Then the dry

residue is ignited at 600°C for 30 mins., cooled in a

desiccator and reweighed. A blank determination was conduc-

ted under the same conditions. Percentage crude fiber was

calculated by determining the loss of weight on ignition

minus the loss in weight of the asbestos blank multiplied

by 100 and divided by the weight of sample.

I. Determination of isothiocyanate and
thiooxazolidones

The isothiocyanate content was determined by the

argentrimetric method of Viehoever et al. (96), and Andre

and Maille (3), in which the mustard oil reacts with ammonia

forming substituted thiourea. The latter decomposes in

ammonical silver nitrate forming insoluble silver sulfide

and monosubstituted carbodiamide. The unreacted silver is

then determined volumetrically by the Volhard method using

potassium thiocyanate solution. The thiooxazolidones were

determined by the method described by Astwood et al. (7)

and modified by Wetter (98).

Samples of the isolates and concentrates weighing approximately 30 mg were mixed with 1.5 ml of 0.5% "crude myrosinase" in 0.9% sodium chloride and 10.0 ml citrate buffer pH 4.0 added and macerated. The contents were then shaken for 2 hrs. at 25°C in a mechanical shaker. The mixture was then distilled in an Aminco distillation apparatus. The distillate, approximately 25 ml was collected in 5 ml 0.1 N silver nitrate containing 1.25 ml 10% ammonium hydroxide, surrounded by an ice and water bath. The control contained only the crude myrosinase solution.

For isothiocyanate

The distillate, in an all glass apparatus, fitted with an air condenser was heated for 30 mins. in a boiling water bath. The contents of the flask was made up of 30 ml after filtration. To the filtrate was added 1.0 ml of 6 N nitric acid and 0.5 ml of 8% ferric ammonium sulfate, and this mixture was titrated to a faint salmon color end point with 0.01 N potassium thiocyanate freshly diluted from an 0.1 N stock solution.

For thiooxazolidones

The suspension after distillation was filtered through Whatmann No. 42 filter paper and the pH of the filtrate was adjusted to 10.5 with 1.0 N sodium hydroxide. One milliliter of this solution was extracted twice with 5.0 ml of anhydrous ethyl ether. The absorbance of the ether layer was measured at 230 nm, 248 nm and 266 nm

against an ethyl ether blank in a Beckman DB spectrophoto-
meter.   The absorbance at 248 nm was corrected by subtracting
the average value of absorbance at 230 nm and 266 nm.

J.   Calibration standard curves for protein determination

A calibration standard curve for the determination
of the protein content of the extracts were prepared by
diluting the extracts from water, acid and base so that
aliquots of these contained different concentrations of pro-
teins from 0.5 to 8.0 mg approximately.

For the absorbance determination 1.0 to 10.1 ml of
the dilute extracts were acidified with 2 drops of 2 N
sulfuric acid and the volume made to 20.0 ml with 3 M urea
after the extracts were made equal in volume.   The contents
were mixed and the absorbance measured at 280 nm in a Beckman
DB spectrophotometer.

Nitrogen was determined by the Kjeldahl method
using the same dilutions and concentrates as for the absor-
bance determination.   The nitrogen was converted to protein
and a plot was made against absorbance.

K.   Slab gel electrophoresis

Slab gel electrophoresis was carried out in
horizontal plates using 10% polyacrylamide gels made in 0.175
M Tris - glycine buffer pH 8.8 containing 4.5 M urea and 0.1
M 2-mercaptoethanol as described by Raymond (74) and Tombs
(91).   The gel plates were preconditioned before application

of samples. The samples of the isolates, concentrates and whey were dissolved in 0.175 M Tris glycine buffer containing 8 M urea and 0.1 M 2-mercaptoethanol and soaked in filter paper strips and applied to slots in the plates. Electrophoresis was carried out for 18 hrs. at 25 mA at 4°C. The plates were stained in 1% amido black in 7% acid for 5 mins. and destained in 7% acetic acid until a clear background was obtained.

L.   Disc gel electrophoresis

Seven percent polyacrylamide gels containing 6 M urea and 0.1 M 2-mercaptoethanol were made in 6 cm long and 0.6 cm internal diameter glass tubes according to the procedure of Davis (26) with a few modifications (63). The samples were taken up in Tris glycine buffer pH 8.6 containing 8 M urea and 0.1 M 2-mercaptoethanol and mixed with a drop of 0.01% bromophenol blue as an internal marker. The electrolytes contained Tris-glycine buffer pH 8.6 and electrophoresis was carried out at 125 volts at a current of 3 milliamperes per tube for 75 mins. The gel tubes after electrophoresis were removed from the glass tubes and stained in 1.0% aqueous coomassie brilliant blue R 250 diluted 1 to 20 with 10.0% trichloroacetic acid before use, after fixing the gels for 30 mins. in 12.5% trichloroacetic acid. The gels were destained in 10% trichloroacetic acid.

M.   Isoelectric focusing

Isoelectric focusing was done according to the methods described by Svensson (88), Vesterberg and Svensson (95), Wrigley (103), Kenrick and Margolis (51) and Catsimpoolas (16) by disc electrophoresis using gels containing ampholine pH 3 to 10, in the presence of urea.   The electrophoresis was done at 250 volts with the current falling from 50 mA to 12 mA during the first 30 mins. and electrophoresis was maintained for a further 60 mins. with 0.4% phosphoric acid in the anodic chamber and 0.5% ethanoldiamine adjusted to pH 10.5 with 1.0 N potassium hydroxide in the cathodic chamber.   The gels after electrophoresis were removed and stained in .2% bromophenol blue solution in ethanol : acetic acid : water (50:5:45 by volume) for 1 hr. and destained by washing in ethanol : acetic acid : ether (30:5:65 by volume) as reported by Awdeh (8).   The gels were destained for 12 to 24 hrs. and were stored in 7% acetic acid for photography.

N.   pH measurement

The gels after isoelectric focusing in duplicate were cut corresponding to the stained bands and were macerated in 0.5 ml water after 4 hrs. the pH was determined using Fisher electrodes -- microprobe combination (Cat. No. 13-639-92) in a Beckman digital pH meter.

In another experiment the gels after isoelectric focusing were cut into 5 mm long sections and suspended in 0.5 ml water.   The pH of these were read after 4 hrs.

O. Amino acid analysis

Hydrolyses of the isolates, concentrates, and whey
were carried out in sealed hydrolysis tubes for 24 hrs. at
110°C with 6 N (constant boiling) hydrochloric acid. The
samples were dissolved in 0.05 N sodium hydroxide and
representative aliquots were freeze dried in the presence of
1.0 ml 0.25 mM norleucine as internal standard. To these
5.0 ml hydrochloric acid were added and frozen and vacuum
was applied after flushing with nitrogen and hydrolyzed in
a force draft oven. The hydrolysate was filtered through a
sintered funnel and evaporated in rotary evaporator repeat-
edly by dissolving in water to remove residual hydrochloric
acid. Finally, the residue was taken in citrate buffer pH 2.2.
The analysis was performed with a Phoenix Micro Amino Analyzer
Model M 6800, Moore-Stein system.

P. Cystine determination

Cystine was determined as cysteic acid following
performic acid oxidation as described by Schram et al. (78)
and Moore (58). Samples of the isolates, concentrates and
whey weighing 2 to 3 mg were mixed with 0.5 ml performic
acid at 0°C and held for 16 hrs. at the same temperature (46).
The excess performic acid was removed by freeze drying using
sodium hydroxide traps. The hydrolysis was performed with
2.5 ml of 6 N hydrochloric acid for 20 hrs. at 110°C in
draft forced oven. The hydrolysate was filtered through
a sintered funnel (fine) to remove any precipitates and

then hydrochloric acid was removed on a rotary evaporator
under reduced pressure at 40°C. The residue was taken up
in 2.0 ml citrate buffer pH 2.2.

Q. Tryptophan determination

The tryptophan was determined by the colorimetric
method of Inglis and Leavers (47), with a few modifications.
To 1 ml of concentrated sulfuric acid, slowly pipetted 0.5
ml of 20% sodium hydroxide in 40% methanol, than 2 mg samples
of the preparations in 0.4 ml water were added, and warmed
for 20 secs. To this mixture add 4 ml glacial acetic acid,
1.0 ml concentrated sulfuric acid with mixing and 2 drops
of 8% potassium persulfate reagent and the absorbance read
at 550 nm after 20 mins. in a Spectronic 20 spectrophoto-
meter. Tryptophan concentration was read off from a
standard tryptophan solution.

The amount of tryptophan calculated by the method
of Edelhock (34) by taking about one third the amount of
tyrosine determined in amino acid analysis agree with the
above determinations.

R. Ultracentrifugation

Ultracentrifugation of the rapeseed protein
isolates P_i was made using a Beckman L2 65B analytical ultra-
centrifuge. All sedimentation analyses were made at 25°C
and 59,000 r.p.m.

The protein isolates for sedimentation analyses

were p repared according to method of Liu (55) by blocking
the sulfhydryl groups, followed by reversible blocking of
amino groups as described by Dixon et al. (28).

To 100 mg protein isolates, 5 g urea and 2 ml 0.1 M
phosphate buffer followed by 4.6 ml water were added and the
pH adjusted to 6.7 with 1 N sodium hydroxide. Then 0.09 ml
of 5% ethylenediamine-tetraacetic acid was added and nitrogen
was bubbled into the mixture for 15 mins. To the mixture was
added 15 μl of 2-mercaptoethanol and incubated for 15 mins.
at 37°C. To the latter reduction mixture was added 150 mg
sodium tetrathionate ($Na_2S_4O_6 \cdot 2H_2O$) and after 5 mins. at
room temperature the pH of the mixture was adjusted to 6.6.
This mixture was dialysed in running water and finally with
demineralized water for 24 hours.

The pH of the dialysed solutions were adjusted to
8 with 1 N sodium hydroxide and treated with 0.83 ml citra-
conic anhydride with stirring at room temperature while
maintaining the pH at 8 by adding 5 N sodium hydroxide. The
base uptake ceased after 10 to 15 mins. The protein solution
was desalted initially by dialysis in running tap water at
4°C followed by demineralized water for 2 days. The dialysed
solutions were then adjusted to pH 8 and after centrifugation
the clear supernatants were freeze dried.

For the sedimentation analyses the freeze dried
samples were dissolved in 0.1 M Tris-glycine buffer pH 8.5.
The concentration of the proteins in solution were 1%.

3. Extraction and preparation of isolates

A. Three stage extraction procedure

The flour was mixed with water at 4°C in the ratio of 1 : 15 and blended for 3 min. using a Lourdes Model NM-1A blender set at 80, (see Plate 2). The mixture was clarified at 10,000 x g for 15 min. at 4°C in a Sorvall RC2-3 centrifuge. The residual meal was re-extracted twice as before. The combined supernatants were filtered.

The residue from the above was extracted twice using 0.1 N and 0.05 N hydrochloric acid at pH of 2 at 60°C as described for the water extract. The residue was further extracted with 0.02 to 0.01 N sodium hydroxide at 40°C at pH 10. After 3 successive extractions supernatants were combined and filtered. The flow diagram for the extraction is given in Figure 1.

B. Single stage sodium hydroxide extraction

The flour was extracted 3 times with 0.01 N sodium hydroxide at pH 10 and 40°C. The mixture was blended for 3 mins. and centrifuged at 10,000 x g for 15 mins. The combined supernatants were filtered. Flow diagram for the extraction is given in Figure 2.

The extracts from the three and single stage procedures, except the second hydrochloric acid extract, were made 0.15% with respect to sodium sulfite before precipitation of isolates or preparations of concentrates.

PLATE II

EXTRACTION APPARATUS - LOURDES BLENDER

Rapeseed flour (15g)

Blend 3 min with
225 ml water at 4°C
and centrifuge

Supernatant (SW)   Residue

Filter, add
0.15% Na_2SO_3
and freeze dry   Blend 3 min with
                                  HCl at pH 2 and 60°C
Concentrate (CW)   and centrifuge

Isolates (P_iW^1 & P_yW^2)

Supernatant (SA)   Residue

Add 0.05 M
oxalic acid and   Blend 3 min with
centrifuge   NaOH at pH 10, and
                             40°C and centrifuge

Supernatant (SA°)   Residue
                          (discard)
Filter and
freeze dry

Concentrate (CA)   Supernatant (SB)   Residue
                                                    (Discard)
                      Filter, add
                      0.15% Na_2SO_3
                      and freeze dry
                   Concentrate (CB

                   Isolates (P_iB^1 & P_tB^2)

1. Supernatants (SW, SA° & SB) $\xrightarrow{\text{Adjust to the isoelectric points}}$
   $\overline{\text{warm to 35°C and centrifuge}}$

   Sediments $\xrightarrow{\text{Wash with water and 50% ethanol}}$ Isolates
   $\overline{\text{dissolve at pH 8.5 and freeze dry}}$ ($P_iW, P_iA°$ & P_iB)

2. Supernatants (SW, SA & SB) $\xrightarrow{\text{Add 12.5% trichloroacetic acid}}$
   $\overline{\text{warm to 35°C and centrifuge}}$

   Sediments $\xrightarrow{\text{Wash with water and 50% ethanol}}$ Isolates
   $\overline{\text{dissolve at pH 8.5 and freeze dry}}$ (P_tW, P_tA & P_tB)

Figure 1.   Flow diagram of the three stage
            extraction procedure

Rapeseed Flour (10 g)

Blend 3 min with
NaOH at pH 10 and
40°C and centrifuge

Supernatant (SS)

Residue
(discard)

Filter, add
0.15% Na_2SO_3

Freeze dry

Adjust pH
5.2, and
warm to 35°C
centrifuge

Add 12.5%
TCA, warm to
35°C and cen-
trifuge

Concentrate
(CS)

Sediment P_iS

Whey
P_iSU

Whey
P_tSU

Sediment P_t

Wash with
water and
50% alcohol
Adjust pH 8.6

Freeze dry

Wash with
water and
50% alcohol
Adjust pH 8.6

Freeze dry

Isolate P_iS

Isolate P_tS

Figure 2.   Flow diagram of the single stage
            sodium hydroxide extraction process.

In another parallel series of experiments the flour before removal of oil was extracted by the three and single stage procedure, as described above. Also the flour was extracted at pH values from 9.5 to 11.5 in order to determine the degree of denaturation of the isolate at the high pH values.

C. Oxalic acid treatment for decreasing ash content in the hydrochloric acid extract (SA)

To 25.0 ml aliquots of the hydrochloric acid extract were added 0.01 to 0.08 M oxalic acid. The solutions were allowed to stand for 1 hr. The precipitate which formed was removed by centrifugation and the resulting supernatant was used to determine the nitrogen by the Kjeldahl method.

The residues left after oxalic acid treatment were ashed and taken up in 0.1 N hydrochloric acid for the calcium determination using the EDTA titration method of Ntailianas and Whitney (61).

D. Preparations for concentrates

The concentrates were obtained from the extracts by freeze drying.

E. Preparation of isolates

The extracts were adjusted to their respective isoelectric points by adding 0.5 N hydrochloric acid or 0.5 N sodium hydroxide and then warmed to 35°C for 10 mins. The precipitates formed were centrifuged for 15 mins. at 15,000 x g and washed twice with water at 50°C, followed by two

washings with 50% ethanol. The washed isolates were

dissolved in water at pH 8.5 and freeze dried (P_i). The

water extract (SW) was adjusted to pH 2.6, and the hydro-

chloric acid extract (SA) to pH 3.6. The third sodium hydro-

xide extract and the single stage sodium hydroxide extract

were adjusted to pH 4.2 and 5.2 respectively.

Aliquots of the extracts were also precipitated by

adding 12.5% trichloroacetic acid and heating to 35°C for 10

mins. The isolates after centrifugation were washed as

described for the isoelectric precipitation, and dissolved

and then freeze dried.

F.   Preparation of whey proteins

The whey obtained after the isoelectric precipita-

tion was freeze dried to obtain the whey protein P_iU.

G.   Preparation of isolates from oxalic acid
treated hydrochloric acid extract (SA°)

The hydrochloric acid extract was made 0.05 M with

oxalic acid and after standing for 1 hr. the mixture was

centrifuged to obtain a supernatant relatively free of

calcium. In one experiment the supernatant was adjusted to

pH 3.6 with 0.5 N sodium hydroxide, and in another, the

extract was adjusted to 12.5% with trichloroacetic acid. The

isolates formed were warmed to 35°C for 10 mins. and separa-

ted by centrifugation at 15,000 x g for 15 mins. The iso-

lates were washed twice with water at 50°C and then twice

with 50% alcohol. The precipitates were then dissolved in

water at pH 8.5 and freeze dried, to obtain isolates P_iA^o and P_tA^o.

H. pH-solubility profiles of the extracts

The determination of nitrogen solubility as a function of pH of the consecutive extraction with water, hydrochloric acid and sodium hydroxide extracts, were carried out as outlined below. To 25 ml aliquots of the 4 extracts were added 0.5 N hydrochloric acid or 0.5 N sodium hydroxide to adjust the pH from 2 to 11. The volumes after pH adjustment were made equal and centrifuged at 15,000 x g for 15 mins. to obtain supernatant whey. The supernatant whey was used for the determination of nitrogen by the Kjeldahl method. Plots were made of the whey nitrogen against the pH of t he respective aliquots.

I. Preparation of "crude myrosinase"

Myrosinase was prepared according to the method described by Wrede (101) from rapeseed meal. Five hundred grams of meal was mixed with 1800 ml of water at 4°C and allowed to stand for 1 hr. before blending. The slurry was centrifuged at 4,000 x g for 15 mins. to separate the supernatant from the residual meal. The supernatant obtained was mixed with an equal volume of 90% ethanol at 4°C and the precipitate formed was centrifuged at 14,000 x g for 15 mins. The precipitate was washed with 7 0% ethanol and centrifuged. The residue was dissolved in 400 ml of distilled water and

then centrifuged to remove any insoluble material to obtain

the soluble fraction which was filtered and lyophilized.

The yield was 0.52% and the preparation was stored at -20°C

until required.

J. Solubility measurements of isolates and concentrates

Solubility of the preparations were determined in

water at 25°C by mixing them thoroughly. These were then

centrifuged at 27,000 x g for 30 mins. and the clear super-

natant was used for the nitrogen determination by the

Kjeldahl method. The percentage of solubility was calcula-

ted for the soluble fraction and in relation to the amount

of sample originally taken for the determination.

CHAPTER IV
RESULTS

A.   Calibration curves for proteins

As seen in Figures 3 and 4, the plot of absorbance against mg nitrogen in the extracts shows a linear relationship.   Each curve has a different gradient due to the different absorbance at 280 nm. The nitrogen content of the extracts was calculated from these curves as a routine procedure.

B.   pH-solubility profile of the extracts

The pH-nitrogen solubility curves are shown in Figures 5 and 6.   From a study of the pH-nitrogen solubility profiles of the rapeseed protein extracts it is seen that the second hydrochloric acid extract (SA) showed 2 minimum points at pH 3.6 and 7.   From these the lower pH showed a larger amount of protein precipitated than the higher pH point.   With the water extract the shape of the curve was not hyperbolic as with the sodium hydroxide extracts, but a minimum point was observed in the region of pH 2.6, with a broad region on either side.   The third sodium hydroxide extract showed a distinct minimum whey nitrogen content at pH 4.2.   The single stage sodium hydroxide extract showed a minimum around the pH 5.2.

C.   Influence of oxalic acid treatment on
     hydrochloric acid extract

A gradual increase in the calcium content in the

Figure 3. Effect of protein concentration of the
first water extract...o...o., and second hydro-
chloric acid extract ---●---●--- on absorbance.
Protein determined by Kjeldahl method and the
absorbance measured at 280 nm in 3 M urea.
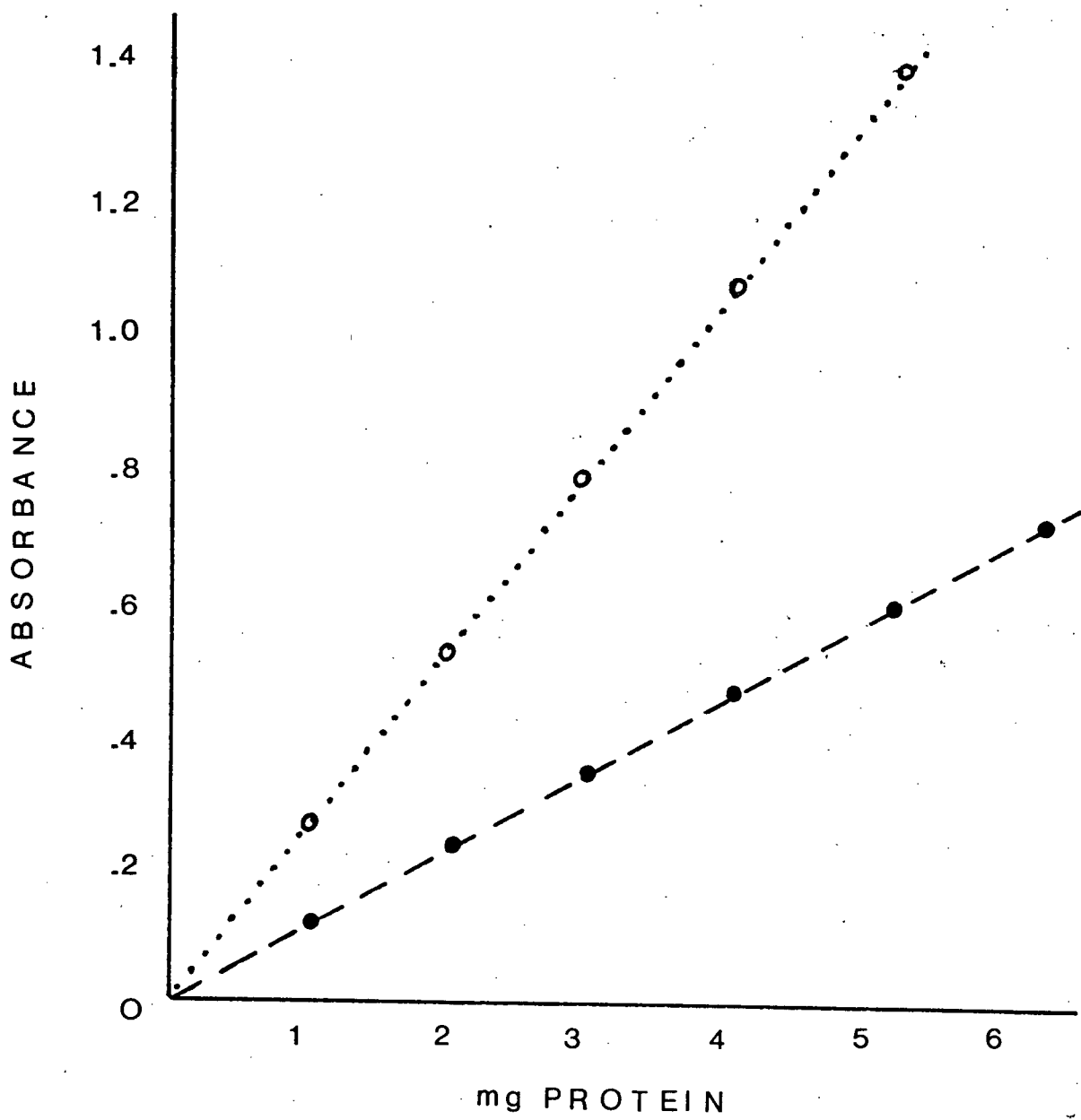
Figure 4. Effect of protein concentration of the
third sodium hydroxide extract -.-o-.-o-.- and
single step sodium hydroxide extract ——o——o-.
Protein was determined by Kjeldahl method and
absorbance measured at 280 nm in 3 M urea.
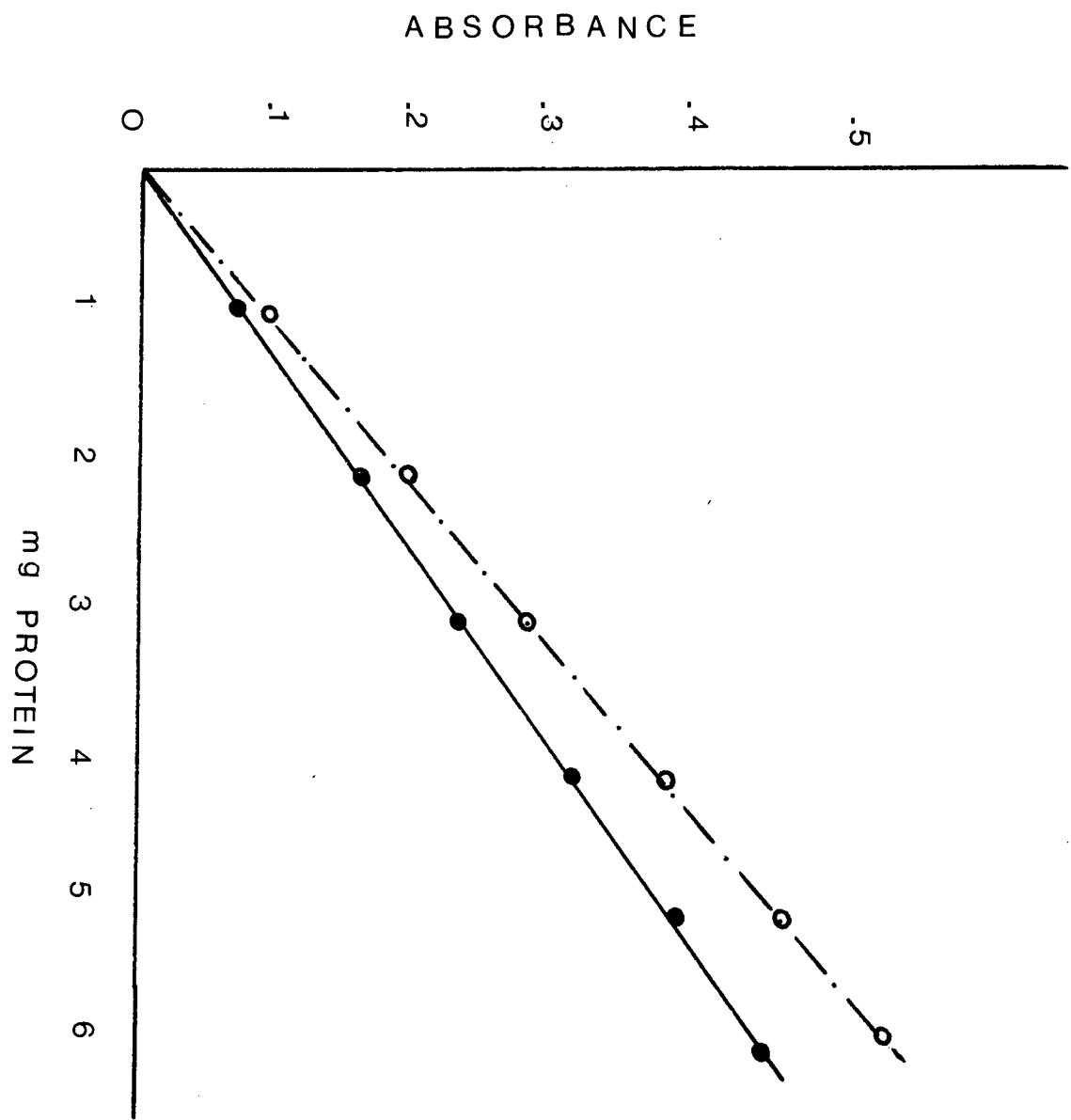
Figure 5. pH-Solubility profiles of the first water
extract ...◉...◉..., and second hydrochloric
acid extract --o--◦.
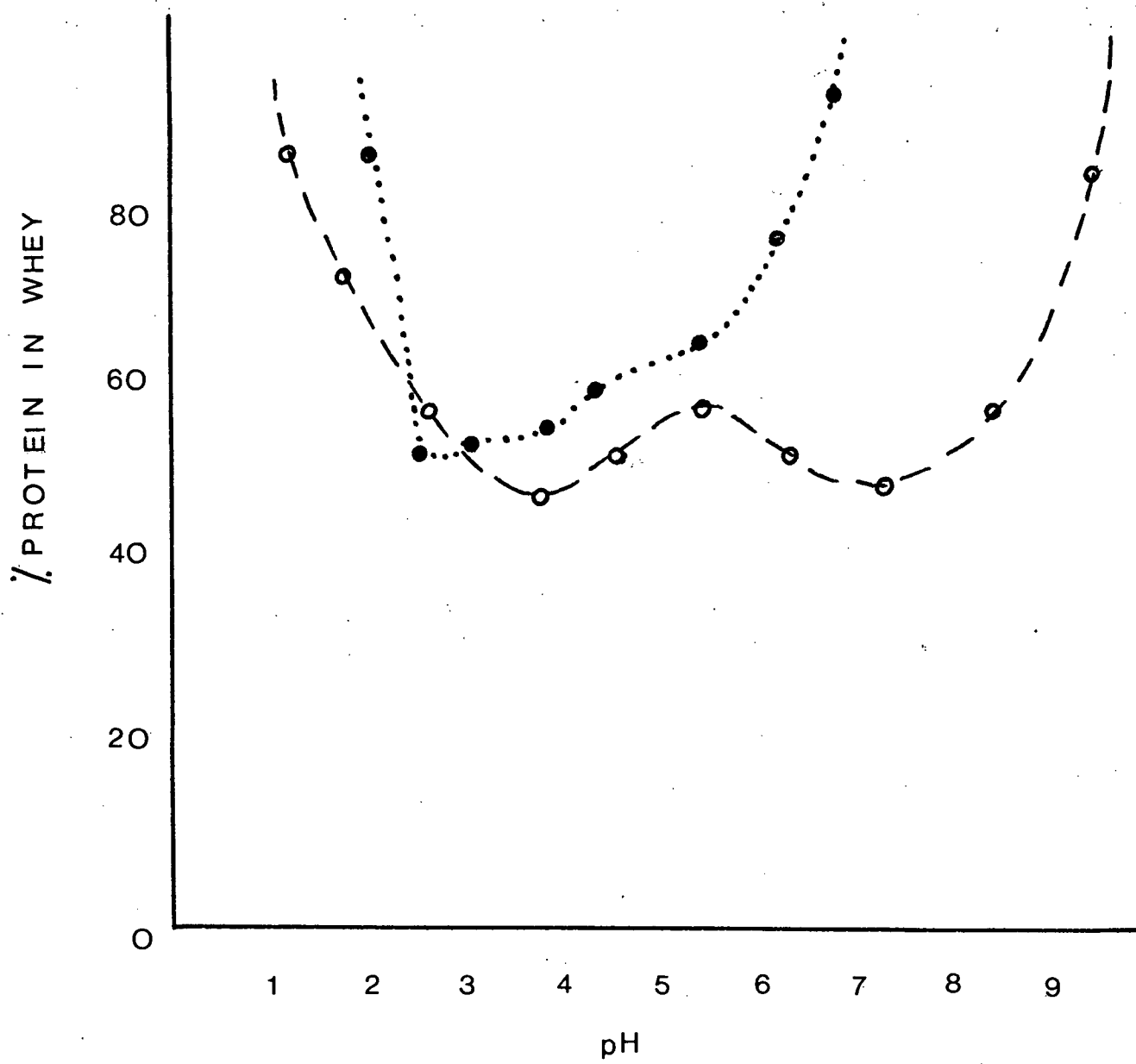
Figure 6. pH-Solubility profiles of the third sodium

hydroxide extract -.-o.-.-, and the single stage

sodium hydroxide extract ——⊘———⊘—.
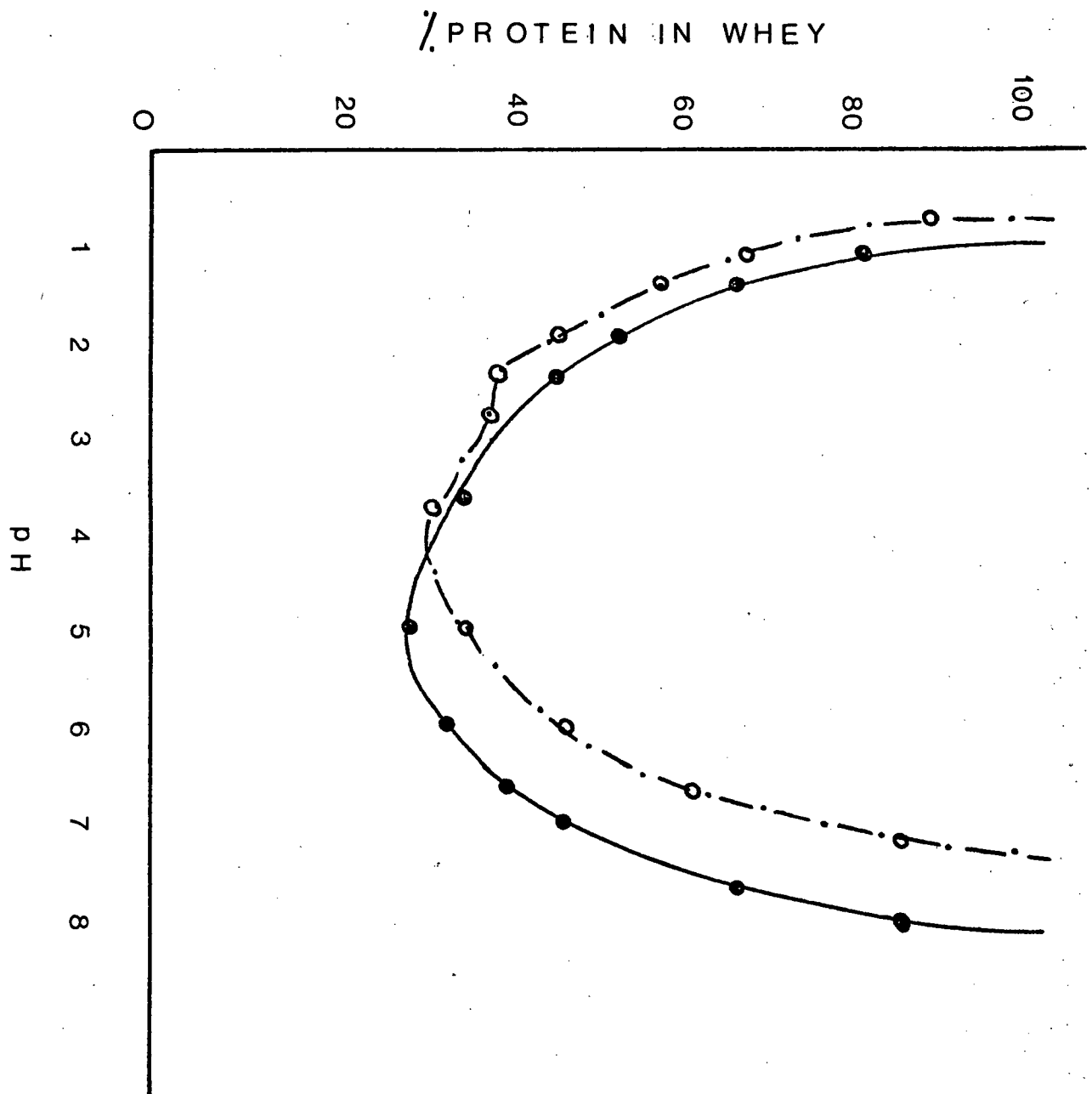
Figure 7. Effect of the oxalic acid treatment of
the second hydrochloric acid extract on
calcium removal.
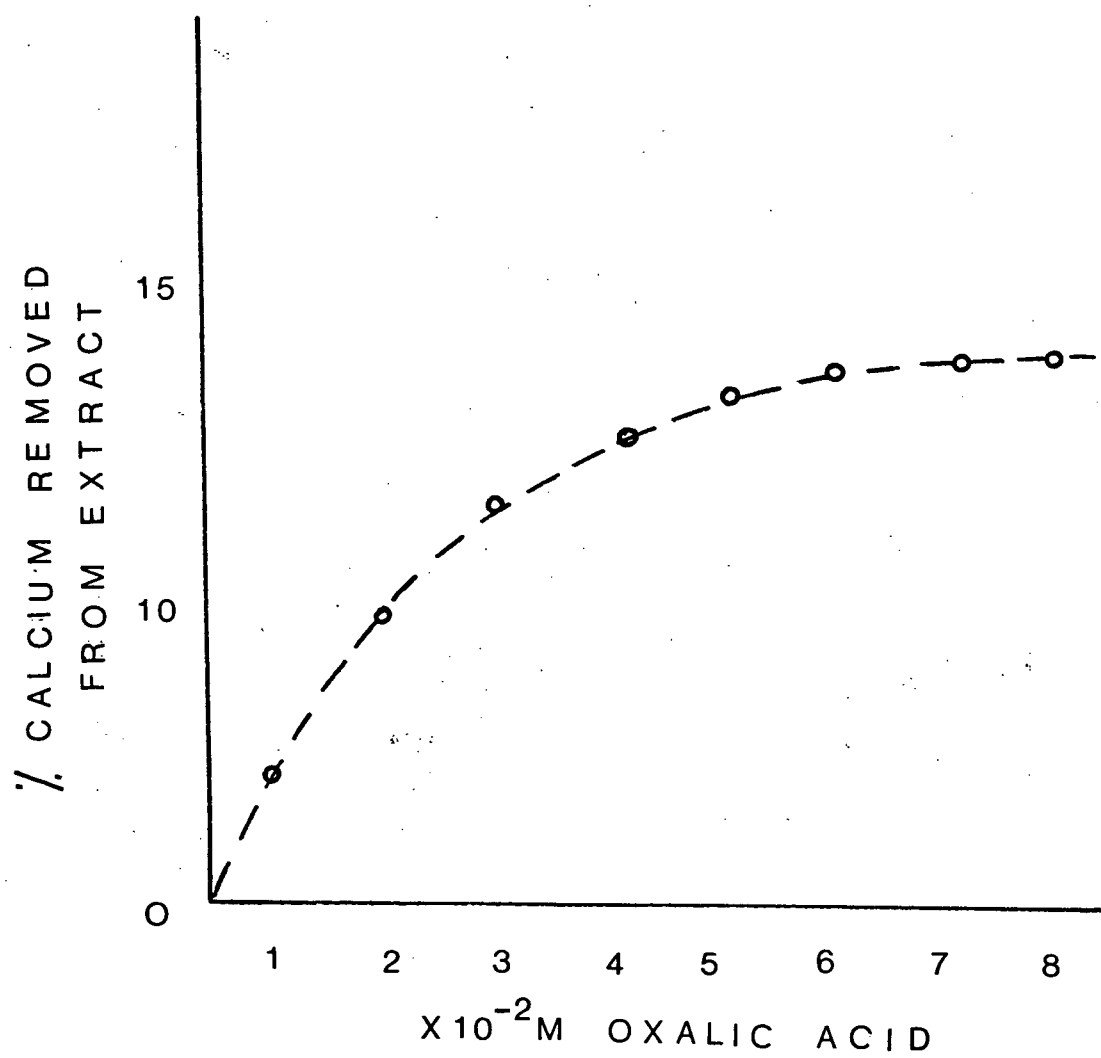
Figure 8. Effect of the oxalic acid treatment of the

second hydrochloric acid extract on protein

content.

Y-axis: % PROTEIN AFTER CALCIUM REMOVAL

X-axis: $X10^{-2}M$ OXALIC ACID
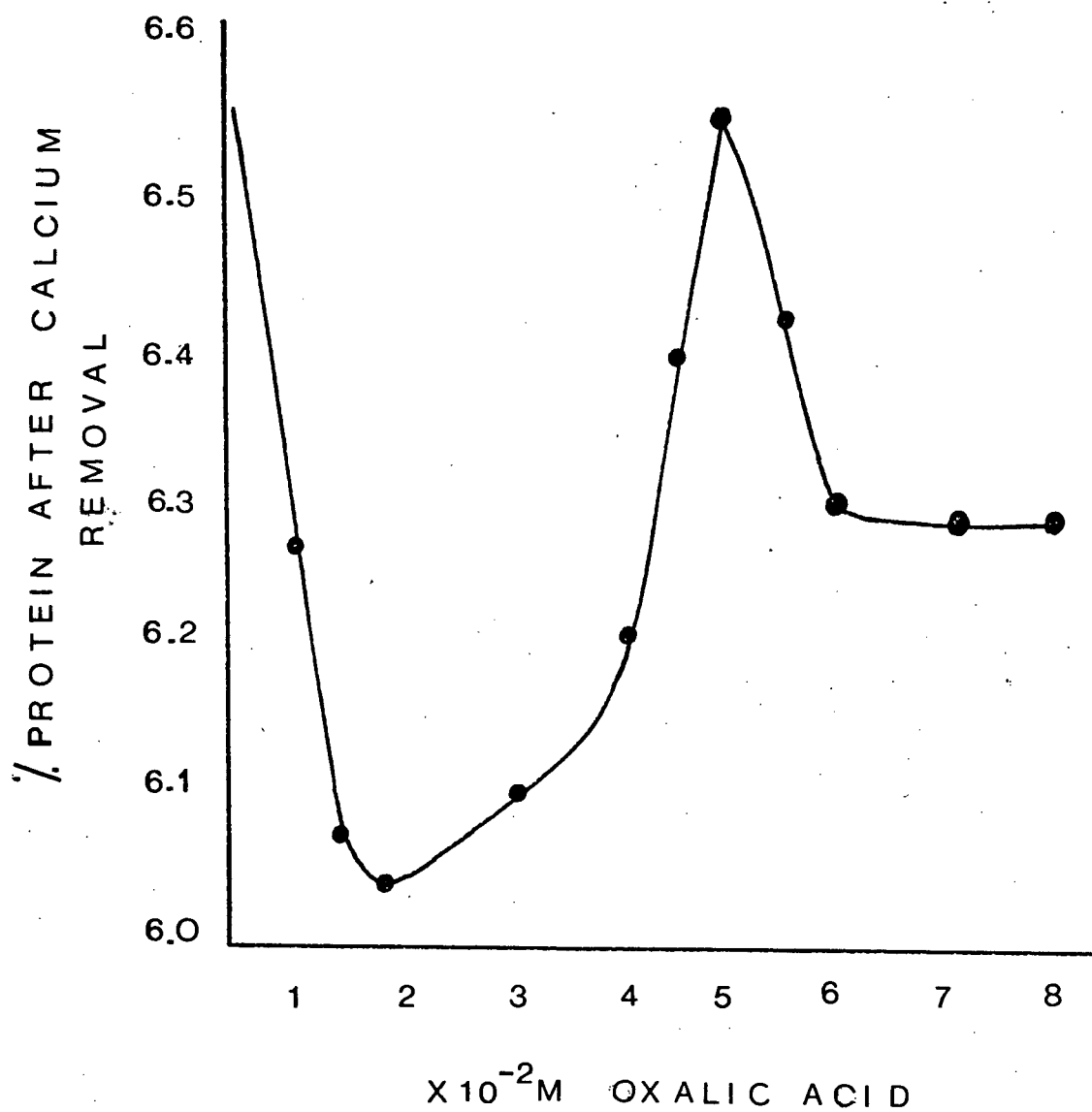
Also with the increasing oxalic acid concentration there was an increase of protein in the supernatant after an initial decrease as illustrated in Figure 8. After reaching the maximum of 0.05 M oxalic acid concentration the protein content decreased again. The residue after addition of oxalic acid and centrifugation contained approximately 9% protein.

           D.   Composition of the protein isolates and
                concentrates

The results are tabulated in Tables I, II and III.

    a.   Moisture determinations

The moisture content of the isolates prepared by isoelectric precipitation P_i ranged from 2.4 to 5.3%, while the range for the isolates P_t was slightly higher, that is 3.2 to 5.6%. Among the isolates the second hydrochloric acid isolates $P_i A$ and $P_t A$ had the highest moisture content.

The concentrates showed moisture levels similar to those of the isolates. The first water and second hydrochloric acid extracts had moisture levels of approximately 6.7%. The two sodium hydroxide extracts concentrates had moisture contents of 3.6%.

    b.   Protein determination

The protein content of the isolates obtained by trichloroacetic acid precipitation (P_t) was slightly higher than that obtained for the isoelectric precipitation (P_i). The value obtained for the second hydrochloric acid

TABLE 1.

PROXIMATE ANALYSIS OF THE RAPESEED PROTEIN
ISOLATES FROM ISOELECTRIC PRECIPITATION

	Moisture	Protein	Carbohydrate	Fat	Ash	Ca	P	S	Crude Fiber	Isothiocyanate
(a)				------% ------						
P_iW	4.7	72.5	2.9	4.2	5.0	0.16	3.31	0.22	1.12	0.89
P_iA	5.3	71.6	1.3	4.8	9.0	1.37	1.82	0.64	1.80	0.55
P_iB	2.3	73.9	2.8	5.8	3.3	1.25	2.50	0.27	1.31	0.30
P_iS	2.4	77.3	3.4	5.2	5.3	0.15	2.52	0.47	1.22	0.65

(a)   For details of abbreviations see pages 32 and 33.

TABLE II

PROXIMATE ANALYSIS OF THE RAPESEED PROTEIN
ISOLATES FROM TRICHLOROACETIC ACID PRECIPITATION

	Moisture	Protein	Carbohydrate	Fat	Ash	Ca	P	S	Crude Fiber	Isothiocyanate
					--- % ---					
P_tW (a)	4.4	83.3	0.7	5.3	1.2	0.73	0.69	0.17	0.85	0.70
P_tA	5.6	78.7	0.9	5.2	1.2	1.12	1.15	0.38	1.67	0.56
P_tB	3.2	81.9	1.1	5.4	1.1	0.84	2.30	0.26	1.24	0.22
P_tS	5.1	82.7	0.6	4.3	1.2	0.49	1.34	0.39	1.33	0.42

(a)   For details of abbreviations see pages 32 and 33.

TABLE III

PROXIMATE ANALYSIS OF RAPESEED PROTEIN
CONCENTRATES

	Mois-ture	Protein	Carbo-hydrate	Fat	Ash	Ca	P	S	Crude Fiber	Isothio-cyanate
					-- --%-----					
CW (a)	6.6	5 2.4	11.3	5.4	14.0	0.61	1.70	0.32	3.35	0.37
CA	6.8	37.3	4.5	4.7	33.6	2.57	6.31	0.11	3.05	0.34
CB	3.6	62.8	3.0	5.9	19.3	0.57	1.90	0.31	2.09	0.20
CS	3.6	63.5	4.1	5.3	16.7	0.74	0.80	0.60	2.21	0.24

(a) For details of abbreviations see pages 32 and 33.

extract isolate P_i was 72% which is low compared to the isolate P_t. The isolate obtained before the oxalic acid treatment was 22% which is far too low for an isolate. All other isolates P_i and P_t had values ranging from 72 to 84%.

Protein content in the concentrates from the second hydrochloric acid and first water extracts were 37 and 52% respectively. The concentrates from the third and single stage sodium hydroxide extracts (CB and CS) had approximately 63% protein.

c.   Lipid determination

The lipid concentration of the isolates P_i ranged from 4.0 to 5.8%, while the trichloroacetic acid precipitation isolates P_tW and P_tA had a slightly greater amount of total lipid, except for the third sodium hydroxide extract isolates P_tB and P_tS which were 5.4 and 4.3% lipid respectively, being lower than for the isolates P_iB and P_iS.

The lipid content of the concentrates were slightly higher than the values obtained for their isolates except for the hydrochloric acid extract.

d.   Carbohydrate content

The carbohydrate content of the isolates P_i were slightly higher than those prepared by trichloroacetic acid precipitation P_t. The carbohydrate content ranged from 1.3 to 3.4%. The isolates P_iS had the largest value of 3.4% while the isolates P_iW and P_iB showed a value of 2.8%. The second stage hydrochloric acid extract isolate P_iA was the

lowest with 1.3%. The trichloroacetic acid precipitated isolates P_t had a carbohydrate content ranging from 0.64 to 1.08%.

Carbohydrate content of the concentrates were higher as expected than that of the isolates. The water concentrate CW had a very high value of 11.3%. The rest of the concentrates ranged from 3.1 to 4.5% which resemble the isolates P_i in their carbohydrate content.

e.   Ash content

The ash content of the isolates P_i varied from 3 to 9%. The ash content of the isolate from the hydrochloric acid extract (P_iA) was decreased by oxalic acid treatment from 36.5 to 9%, which was still higher than other isolates. The ash content of the isolates P_t were approximately 1.1% and the oxalic acid treatment increased it for isolate P_iA° to 2.0%. Therefore the trichloroacetic acid precipitation was done without oxalic acid treatment for the hydrochloric acid extract.

The ash content of the concentrates were higher, between 14 to 19% except 34% for the hydrochloric acid extract (CA). The ash content for the single stage sodium hydroxide extract was 16.7%.

f.   Calcium content

The calcium content of the isolates P_i ranged from 0.14 to 1.4%. The calcium content of the oxalic acid treated hydrochloric acid extract P_iA° had the highest value of 1.4%.

The trichloroacetic acid precipitated protein isolates P_t contained 0.5 to 1.1% calcium. The isolate P_tS showed the low value of 0.5%. The calcium content of the hydrochloric acid extract before oxalic acid treatment was approximately 2.6%.

The concentrates had a calcium content ranging from 0.6 to 0.8% except for the hydrochloric acid extract (CA) which was 2.6% similar to the isolate P_iA.

g.   Total sulfur

The sulfur content of the isoelectric precipitated isolates P_i was higher than that of the trichloroacetic acid precipitated isolates P_t. The isolate $P_iA°$ had a concentration of 0.64% which is nearly twice that present in the isolate P_tA. The isolates P_iB, P_iW and P_tW had lower values of 0.24, 0.22 and 0.17% respectively. The single stage sodium hydroxide extract isolates P_iS and P_tS had 0.47 and 0.39% respectively.

The amount of sulfur present in the concentrates was quite similar to that in the isolates, except concentrates CS and CA having 0.60 and 0.11% compared to 0.47 and 0.64% in the respective isolates P_iS and $P_iA°$. Thus the sulfur content in the concentrate CA is very much less than that present in the isolate $P_iA°$. The concentrates CW and CB had a sulfur content of 0.3%.

h.   Phosphorus content

The phosphorus content of the isolates P_i and P_t

vary from 0.7 to 3.3%. The amount of phosphorus in the iso-
lates P_t was lower than the isolates P_i. The isolates $P_iA°$
and P_iA were 1.8 and 11.6% phosphorus with and without oxalic
acid treatment respectively. The highest value was in the
isolate P_iW. The trichloroacetic acid precipitated isolate
P_tB also had a high value of 2.3% phosphorus. The amount of
the phosphorus in the third sodium hydroxide extract isolates
P_iB and P_tB did not show much of a difference as noted for
the other isolates.

The concentrates had phosphorus contents similar
to the isolates. The highest amount among the concentrates,
6.3% was observed in the preparation CA. The other concen-
trates ranged from 0.9 to 2.0%. The single stage sodium
hydroxide extract concentrate CS had the lowest value and
was close to that obtained by Lo and Hill (1971) with a con-
centration of 1.1%.

i.   Crude fiber

Crude fiber content of the isolates P_t ranged from
0.9 to 1.1%, while the isolates P_i had a slightly higher
range from 1.1 to 1.8%. The second stage hydrochloric acid
extract isolate $P_iA°$ had the highest value 1.8%. Published
data for soybean protein isolates is 0.7%.

The concentrates had a crude fiber content slightly
higher, ranging from 2.3 to 3.7%. The water extract concen-
trate CW had the value of 3.7% while the hydrochloric acid
extract concentrate had 3.1%. The soybean protein concen-

trates were reported to contain 3%.

j. Isothiocyanate and thiooxazolidone content

The isothiocyanate content of the isolates prepared by isoelectric precipitation P_i had higher amounts than the trichloroacetic acid precipitated isolates P_t, except the hydrochloric acid extract isolates. The first water extract isolate $P_i W$ had a high value of 0.9%, while the third basic extract isolates $P_i B$ and $P_t B$ had values of 0.32 and 0.22% respectively for both which are the lowest values obtained. The isolates $P_i S$ and $P_t S$ had values of 0.65 and 0.42% isothiocyanate respectively.

The concentrates had a lower range for the isothio-cyanate content was from 0.2 to 0.37%. The water extract concentrate had the highest value of 0.37%.

There was no detectable thiooxazolidone present in the isolates and concentrates present by the method used.

E. Electrophoresis

(a) Slab gel

As indicated slab gel electrophoresis of isolates P_i in Figure 9, most of the acidic and neutral proteins were extracted within the first stage whereas the second stage hydrochloric acid extracted only the basic protein fractions. The residual fractions appear to be extracted in the third sodium hydroxide extract, of which pattern was similar to that of the single stage sodium hydroxide extract. The isolate

Figure 9. Slab gel electrophoretic pattern of the
rapeseed protein isolates prepared by isoelectric
precipitation. Stained in 0.2% Amido black in
7% acetic acid.

W - first water extract isolate P_iW

A - second hydrochloric acid extract isolate P_iA

B - third sodium hydroxide extract isolate P_iB

S - single stage NaOH extract isolate P_iS.
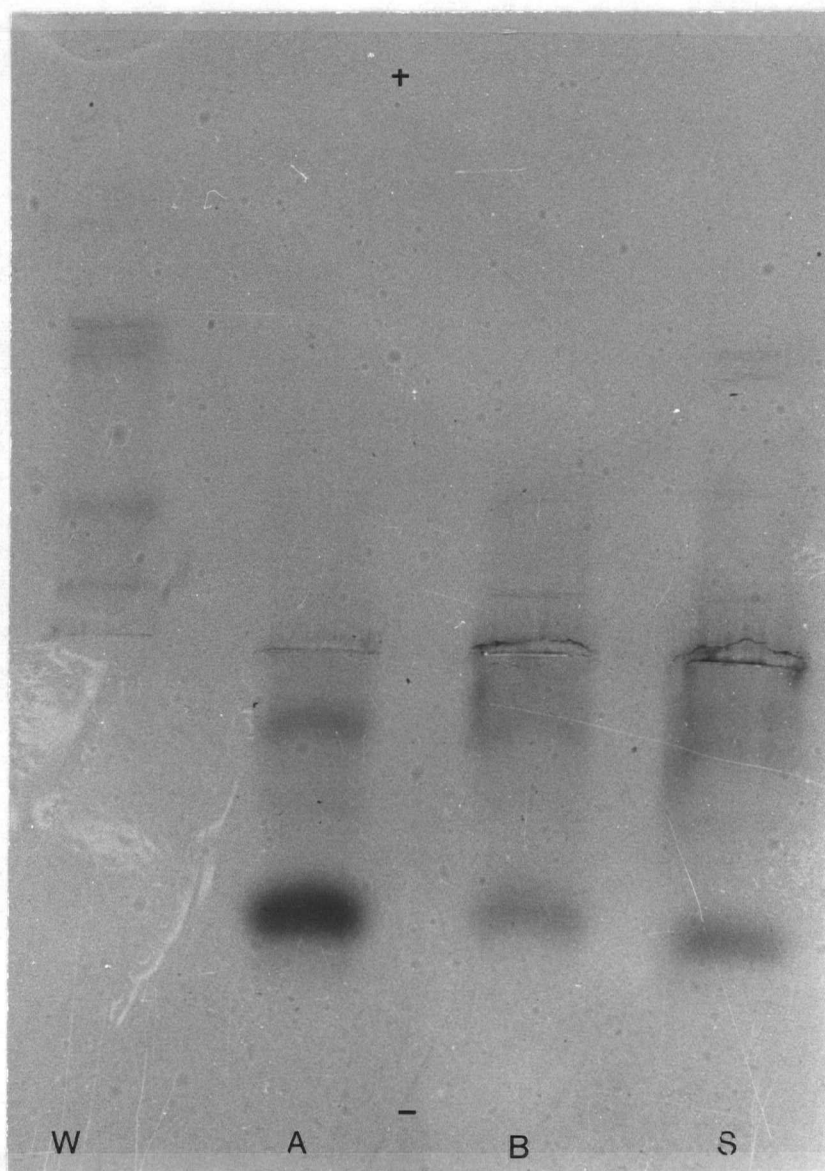
Figure 10. Slab gel electrophoretic pattern of the
rapeseed protein whey (U) after isoelectric
precipitation, P_iU. Stained with 0.2% Amido
black in 7% acetic acid and destained in 5%
acetic acid.

W - first water extract isolate whey P_iWU

A - second hydrochloric acid extract isolate whey P_iAU

B - third sodium hydroxide extract isolate whey P_iBU

S - single stage NaOH extract isolate whey P_iSU.
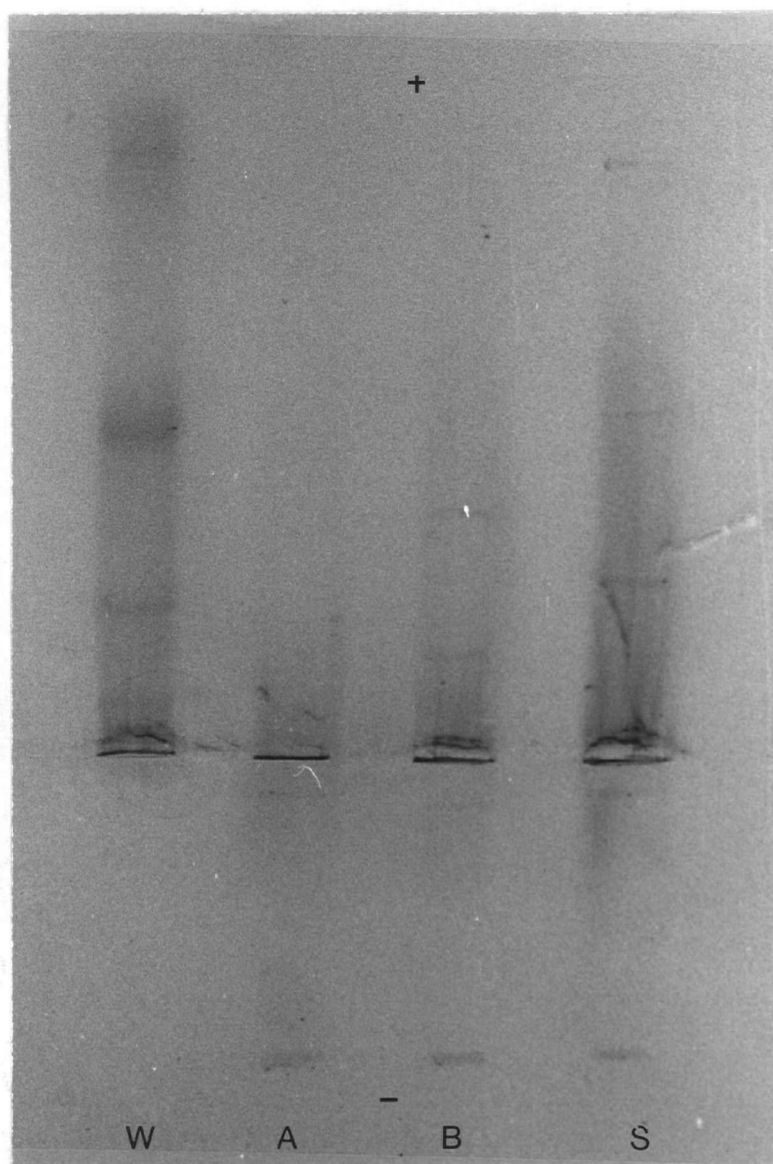
P_t and concentrates showed similar patterns.

The whey from the isolates obtained after isoelectric precipitation showed a number of bands corresponding to their isolates. This is shown in Figure 10. The whey from the water extract tends to show a trailing effect.

(b) Disc gel

Disc gel electrophoresis of the isolates P_i and P_t and the concentrates are shown in Figures 11, 12 and 13. The electrophoresis patterns for the different preparations show a distinct number of bands. Thus for example the water extract isolates and concentrate show a similar separation of protein components. This is true for all the extracts that were made.

The whey protein isolates shown in Figure 14 also show a similar pattern to that of the isolate P_i.

F. Isoelectric focusing

The pH range 3 to 10 for electrofocusing enables one to obtain a complete isoelectric spectrum of both acidic and basic proteins.

It was seen from these experiments as indicated in Figures 15, 16 and 17 that the majority of the protein isolates P_iA^o, P_iB and P_iS have isoelectric points between pH 7 and 10, except for the isolate P_iW which lies between 3 and 7. This picture was true for the isolate P_t and the concentrates. As seen in these figures some of the proteins precipitated during electrophoresis and migrated to the acidic side at the top of the column. A fair amount of

Figure 11. Disc gel electrophoretic pattern of the
    rapeseed protein isolates prepared by isoelectric
    precipitation. Buffer: Tris glycine, pH 8.6.
    Strained with coomassie brilliant blue R 250 in
    10% trichloroacetic acid.
    W - first water extract isolate $P_i W$
    A - second hydrochloric acid extract isolate $P_i A$
    B - third sodium hydroxide extract isolate $P_i B$
    S - single stage NaOH extract isolate $P_i S$.

−

W A + B S
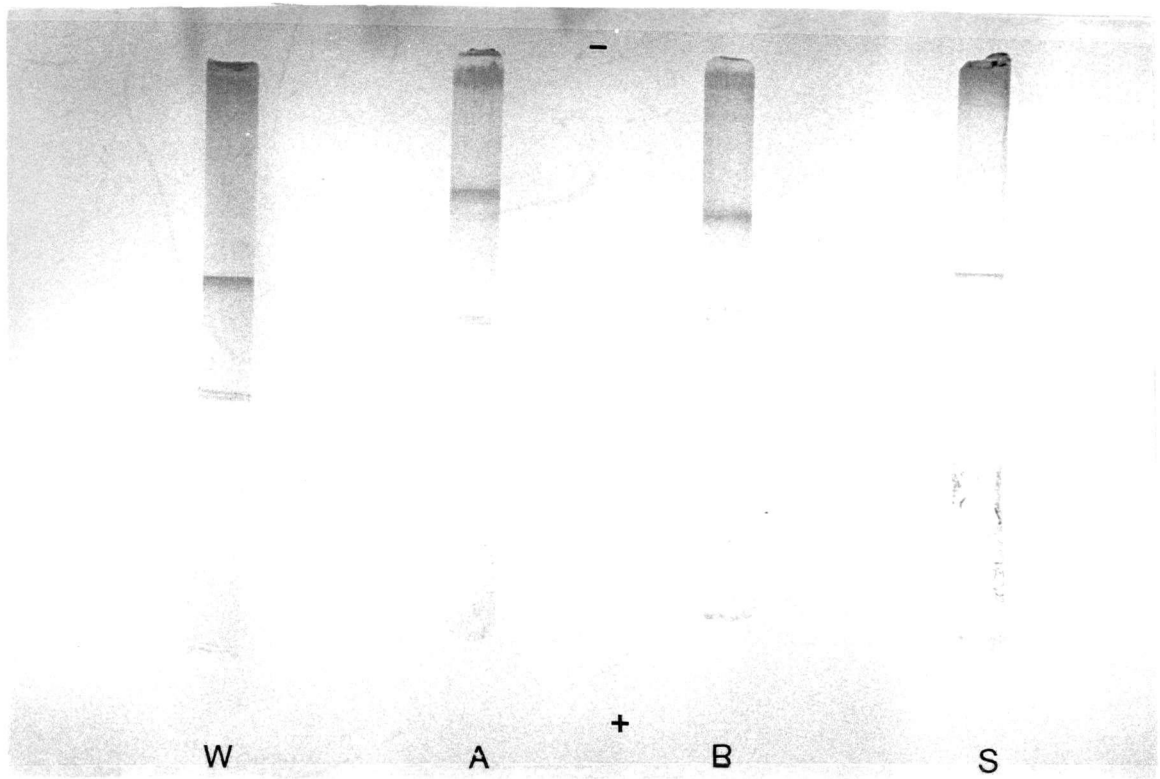
Figure 12. Disc gel electrophoretic pattern of the rapeseed protein isolates prepared by trichloroacetic acid precipitation. Buffer: Tris glycine, pH 8.6. Stained with coomassie brilliant blue R 250 in 10% trichloroacetic acid.

W - first water extract isolate P_tW

A - second hydrochloric acid extract isolate P_tA

B - third sodium hydroxide extract isolate P_tB

S - single stage NaOH extract isolate P_tS.
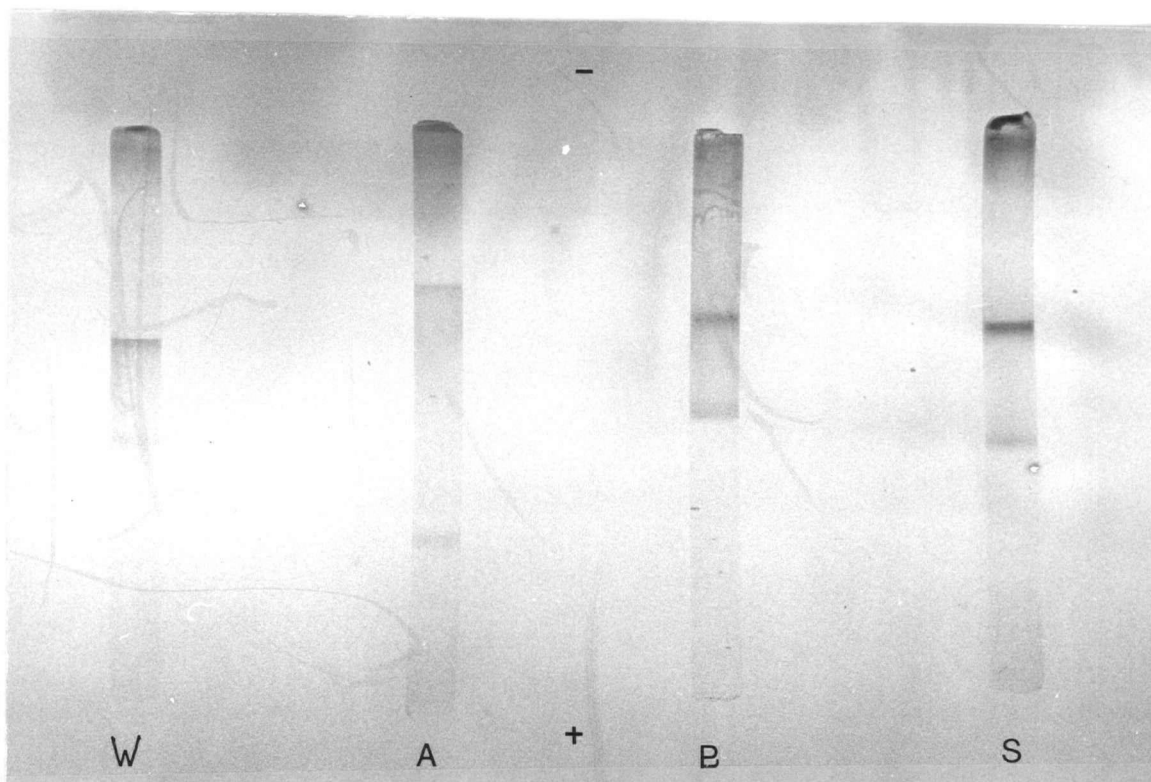
Figure 13. Disc gel electrophoretic pattern of the

rapeseed protein concentrates. Buffer: Tris

glycine, pH 8.6. Stained with coomassie brilliant

blue R 250, in 10% trichloroacetic acid.

W - first water extract concentrate CW

A - second hydrochloric acid extract concentrate CA

B - third sodium hydroxide extract concentrate CB

S - single stage NaOH extract concentrate CS.

W   A   B   S
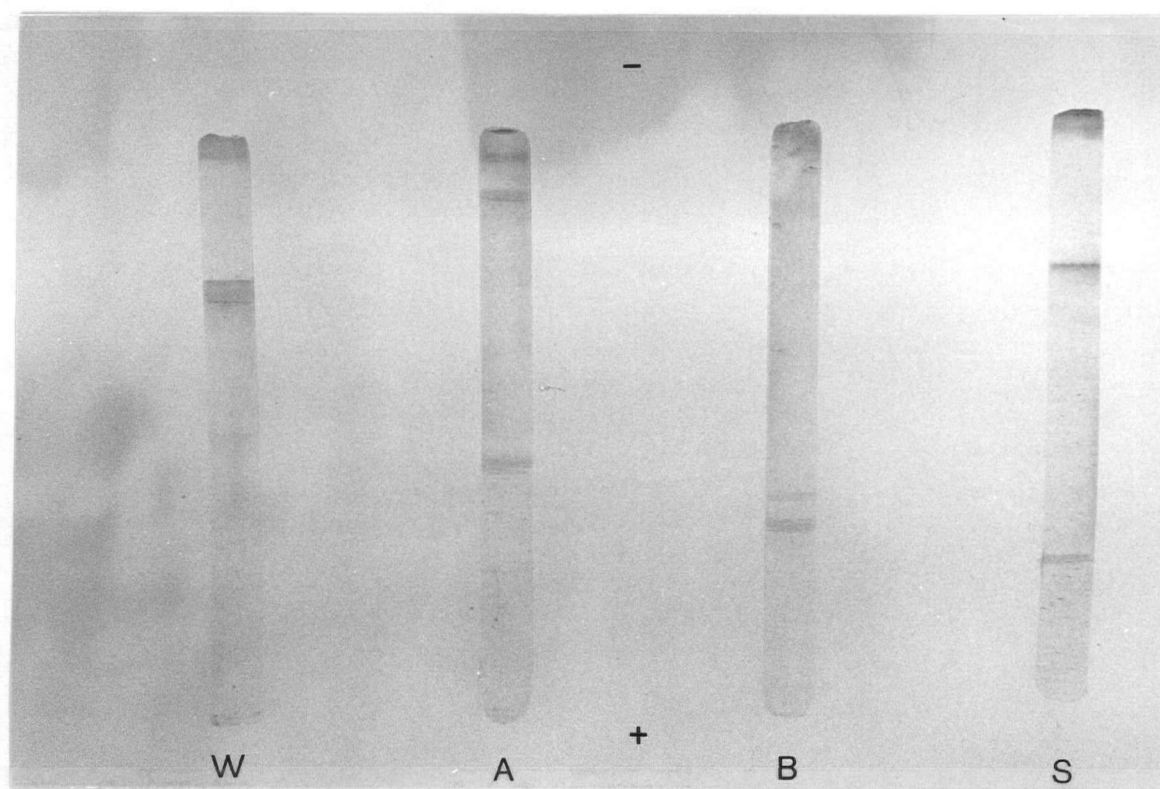
Figure 14. Disc gel electrophoretic pattern of the

rapeseed protein whey after isoelectric precipi-

tation. Buffer: Tris glycine, pH 8.6. Stained

with coomassie brilliant blue R 250 in 10%

trichloroacetic acid.

W - first water extract whey P_iWU

A - second hydrochloric acid extract whey P_iAU

B - third sodium hydroxide extract whey P_iBU

S - single stage NaOH extract whey P_iSU.
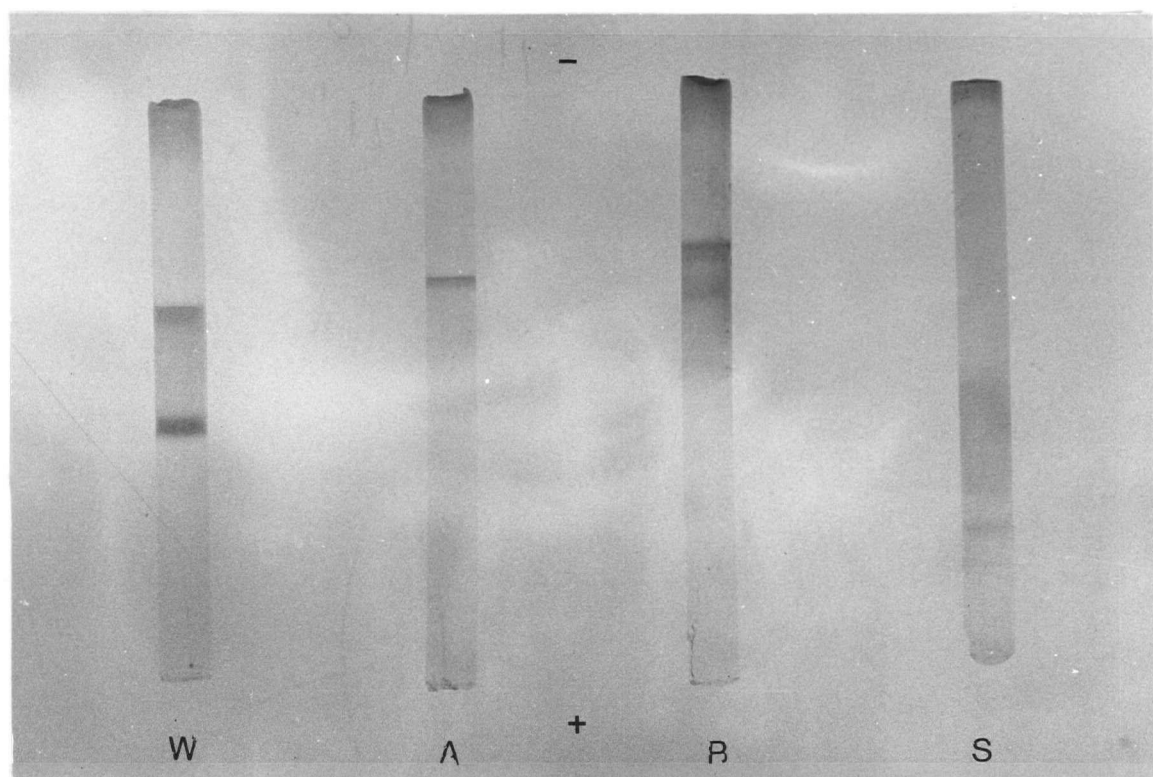
Figure 15. Isoelectric focusing of the rapeseed protein

isolates prepared by isoelectric precipitation, at

pH range 3 and 10. Stained in 0.2% bromophenol blue.

W - first water extract isolate P_iW

A - second hydrochloric acid extract isolate P_iA

B - third sodium hydroxide extract isolate P_iB

S - single stage NaOH extract isolate P_iS.
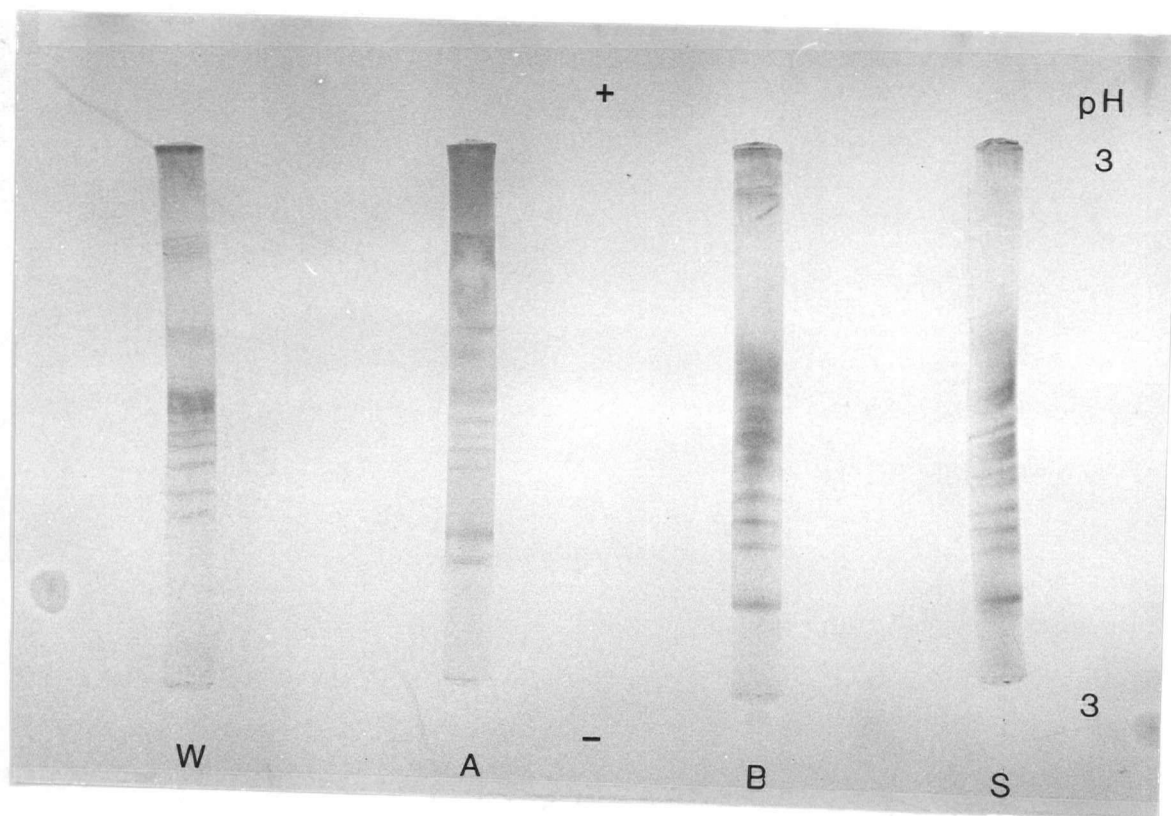
Figure 16. Isoelectric focusing of the rapeseed protein

isolates prepared by trichloroacetic acid precipita-

tion, at pH range 3 and 10. Stained in 0.2% bromo-

phenol blue.

W - first water extract isolate P_tW

A - second hydrochloric acid extract isolate P_tA

B - third sodium hydroxide extract isolate P_tB

S - single stage NaOH extract isolate P_tS.
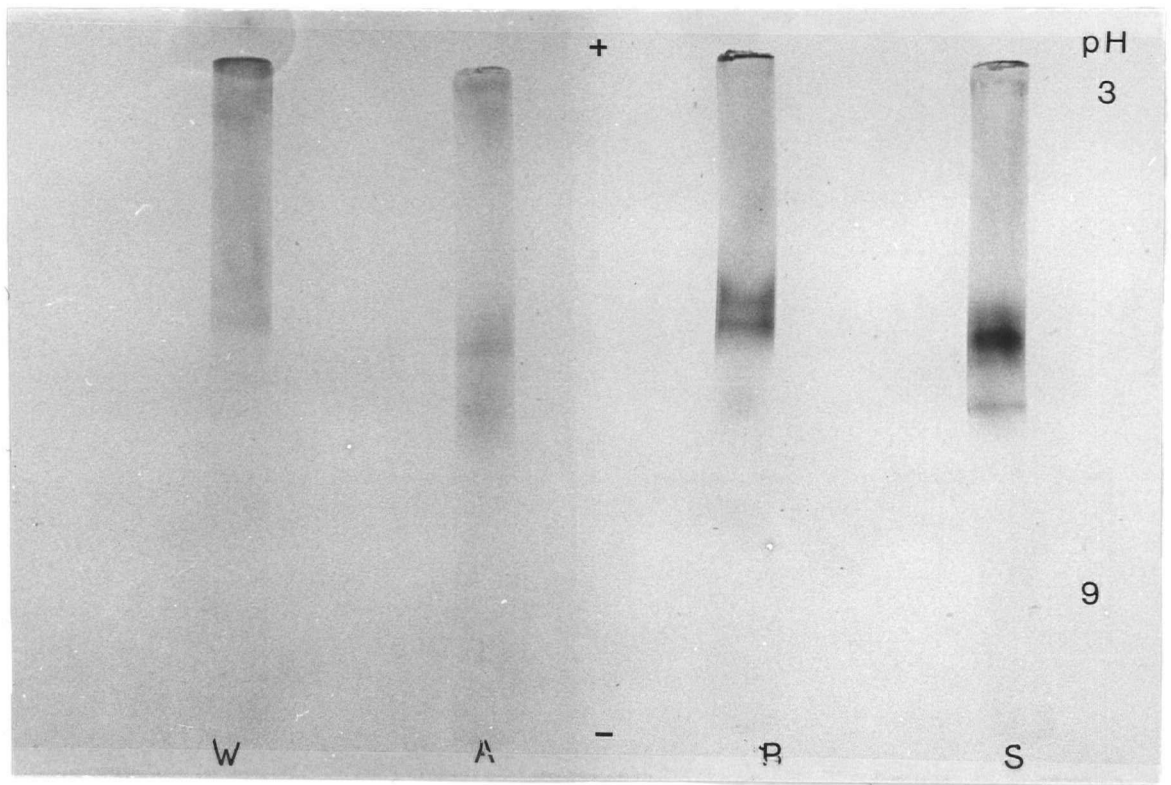
Figure 17. Isoelectric focusing of the rapeseed protein

concentrates, at pH range 3 and 10. Stained in 0.2%

bromophenol blue.

W - first water extract concentrate CW

A - second hydrochloric a cid extract concentrate CA

B - third sodium hydroxide extract concentrate CB

S - single stage NaOH extract concentrate CS.
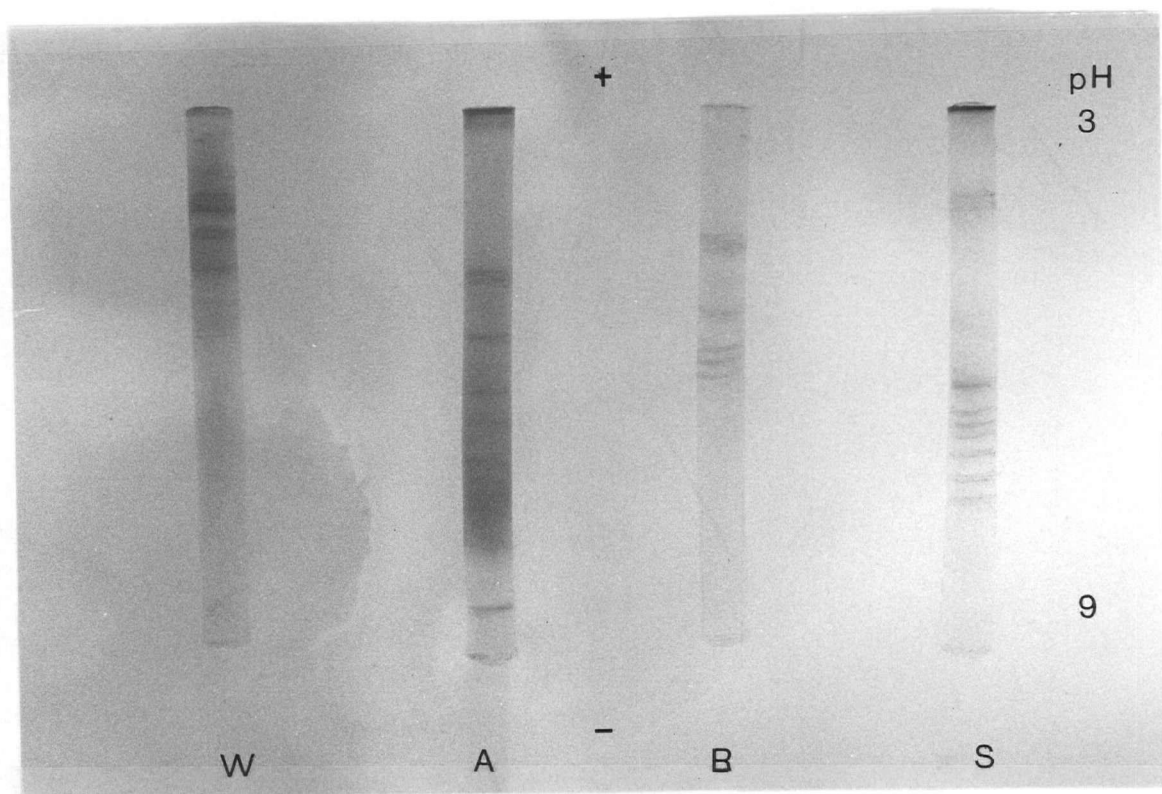
protein, particularly from the acidic and basic extracts,
migrate to the basic side at the bottom of the column.

The results for the whey proteins showed similar
patterns as for the isolates.

When isoelectric focusing was carried in gel con-
taining no urea especially the hydrochloric acid extract
isolates tend to remain at the top of the column.

G. Amino acid composition

The amino acid composition of the isolates and con-
centrates are given in Tables 4, 5, 6 and 7. In the concen-
trates CW and CA there were higher levels of glutamic acid,
cystine and methionine, 30 and 45%, 33 and 45% and 22 and 21%
more than in the isolates P_iW and P_iA^o respectively. In
addition CA had a 70% more proline than the isolate P_iA^o,
while P_iB had 15% lower proline than in the concentrate CB.
The isolates P_iB and P_iS revealed a lower level of glutamic
acid, methionine and phenylalanine, however, 35 and 25%
39 and 25% and 20 and 25% less than those in the concen-
trates CB and CS respectively. The isolate P_iS also had
a 3 8% higher proline content than the concentrates CS.
The r emaning amino acids were higher in the isolate.

The trichloroacetic acid precipitated isolates P_t
had a similar pattern as the isoelectric precipitated
isolates P_i.

The isolates P_iW and P_iA^o indicated higher lysine,
arginine, methionine and proline for the water extract, and
threonine, aspartic acid, leucine and tyrosine for the

TABLE IV

AMINO ACID COMPOSITION OF RAPESEED PROTEIN CONCEN-
TRATES FROM THE DIFFERENT FRACTIONS COMPARED TO
THAT OF RAPESEED AND SOYBEAN PROTEIN CONCENTRATES
(G AMINO ACID PER 16 G NITROGEN)

| | Three stage extraction | | | Single | Rapeseed[a] | Soybean[b] |
	Water	HCl	NaOH	NaOH extract		
Asp.	6.9	4.1	6.7	6.3	6.0	11.3
Thr.	6.8	4.5	5.1	5.3	3.6	3.9
Ser.	4.6	4.9	5.0	4.9	3.7	6.0
Glu.	17.0	19.5	19.3	15.9	16.4	18.5
Pro.	3.4	4.9	5.3	4.8	5.6	4.0
Gly.	6.8	5.7	5.1	5.4	4.6	4.6
Ala.	6.2	5.2	5.0	6.0	4.0	5.3
Cys.#	1.3	2.7	2.4	2.4	1.9	1.3
Val.	4.1	4.5	5.3	5.0	4.6	5.6
Met.#	2.1	2.5	3.1	2.5	1.6	1.3
Ile.	3.0	3.8	4.4	4.0	3.8	5.3
Leu.	5.5	7.0	8.0	7.2	6.8	8.1
Tyr.	3.8	3.0	3.5	3.3	2.3	4.4
Phe.	2.2	3.7	4.2	3.9	3.7	5.6
Lys.	6.9	6.6	4.6	6.6	6.2	6.7
His.	5.1	3.5	2.1	3.4	2.7	2.6
Arg.	4.8	5.5	3.7	5.0	5.8	6.5
Try.*	1.4	1.1	1.3	1.2	0.8	1.4
Amm.	2.4	2.2	1.6	2.1	–	–
Total	94.3	95.3	95.9	95.5	84.6	82.7

\#   Performic acid oxidation separate determinations, by the
    method of Schram et al. (1954) and Moore (1963).

*   Tryptophan determined by the method of Inglis and Leavers
    (1964).

a. Values from Lo and Hill (1971).

b. Values from Van Etten et al. (1959).

TABLE V

AMINO ACID COMPOSITION OF RAPESEED PROTEIN ISOLATES
PREPARED BY ISOELECTRIC PRECIPITATION COMPARED TO
SOYBEAN PROTEIN ISOLATES AND RAPESEED PREPARATION B1
(G AMINO ACID PER 16 G NITROGEN)

	Three stage extraction			Single NaOH extract	Soybean protein[a]	B1.[b]
	Water	HCl	NaOH			
Asp.	7.1	6.5	6.2	6.2	10.5	10.4
Thr.	5.3	7.2	5.4	5.7	3.5	4.2
Ser.	4.5	5.2	4.4	4.6	4.7	4.0
Glu.	11.9	10.9	12.5	12.0	17.6	22.2
Pro.	2.3	2.9	4.5	3.5	5.1	5.0
Gly.	5.4	5.9	5.5	5.1	3.9	5.8
Ala.	6.3	5.9	5.1	5.4	4.1	4.5
Cys.#	0.9	1.5	2.4	2.0	1.5	1.8
Val.	5.0	5.3	5.3	4.9	4.8	5.3
Met.#	1.7	2.0	1.9	1.9	1.5	1.3
Ile.	3.5	4.5	4.0	3.5	4.6	4.3
Leu.	6.3	8.5	7.1	6.5	7.8	8.0
Tyr.	3.9	4.8	3.2	3.4	3.5	2.8
Phe.	2.6	3.4	3.4	2.9	5.1	5.1
Lys.	9.1	7.2	5.0	8.6	6.2	3.2
His.	5.5	5.6	4.2	5.8	2.4	1.9
Arg.	7.8	8.7	6.7	9.7	6.0	7.6
Try.*	1.5	1.8	1.2	1.3	1.2	0.7
Amm.	2.0	2.4	2.4	1.9	–	2.1
Total	91.1	97.1	87.4	92.6	93.9	95.9

Performic acid oxidation separate determinations, by the methods of Schram et al. (1954) and Moore (1963).

* Tryptophan determined by the method of Inglis and Leavers (1964).

a. Values from Lo and Hill (1971) for Assay protein C-1 Skidmore Enterprises.

b. Values from Finlayson et al. (1968).

TABLE VI

AMINO ACID COMPOSITION OF RAPESEED PROTEIN ISOLATES
PREPARED BY TCA PRECIPITATION COMPARED TO CASEIN AND
RAPESEED FLOUR (G AMINO ACID PER 16 G NITROGEN)

	Three stage extraction			Single NaOH extract	Casein[a]	Rapeseed flour[b]
	Water	HCl	NaOH			
Asp.	7.2	5.3	6.1	6.1	7.2	6.4
Thr.	7.2	5.8	5.9	5.6	4.9	3.0
Ser.	4.3	4.7	4.5	4.0	6.4	4.1
Glu.	14.9	13.6	14.3	14.0	22.7	17.6
Pro.	4.6	4.4	5.9	5.1	11.5	5.1
Gly.	6.9	5.4	5.6	5.6	2.7	4.4
Ala.	6.7	5.5	5.0	5.3	3.0	3.8
Cys.#	0.7	3.2	0.9	2.4	0.3	0.9
Val.	4.4	4.7	5.3	5.2	7.3	2.5
Met.#	1.2	2.2	0.9	2.3	2.8	1.7
Ile.	3.3	3.6	4.0	3.8	6.1	1.9
Leu.	6.5	7.3	7.5	7.2	9.3	5.3
Tyr.	4.0	3.5	3.4	4.6	6.4	2.2
Phe.	2.3	3.5	3.4	3.1	5.0	3.3
Lys.	8.3	7.5	6.7	7.6	8.3	4.4
His.	4.5	5.8	4.8	5.6	3.1	2.2
Arg.	6.0	8.5	7.5	7.8	4.1	3.8
Try.*	1.5	1.3	1.3	1.7	2.1	–
Amm.	1.6	1.5	2.6	2.3	–	–
Total	94.0	93.4	94.7	95.3	113.1	72.6

\# Performic acid oxidation separate determinations, by the
methods of Schram et al. (1954) and Moore (1963).

* Tryptophan by the method of Inglis and Leavers (1964).

a. Values for casein, Sheffield Chem. Norwick, N.Y. from
Lo and Hill (1971).

b. Values from Tape et al. (1970).

TABLE VII

AMINO ACID COMPOSITION OF RAPESEED PROTEIN WHEY
AFTER ISOELECTRIC PRECIPITATION, AND THOSE OF
SOYBEAN PROTEIN WHEY (G AMINO ACID PER 16 G
NITROGEN)

	Three stage extraction			Single NaOH extract	Soy whey[a]	FAO[b]
	Water	HCl	NaOH			
Asp.	7.1	2.2	2.2	4.2	13.9	
Thr.	7.3	2.8	3.8	5.1	5.4	2.8
Ser.	5.1	5.2	3.4	4.7	6.7	
Glu.	16.6	20.7	28.9	23.5	14.6	
Pro.	4.6	5.6	8.9	8.0	5.4	
Gly.	8.4	6.1	4.1	6.3	4.7	
Ala.	6.6	5.5	3.5	5.0	5.2	
Cys.#	1.7	5.3	2.7	11.1	2.3	
Val.	3.6	3.9	5.1	4.2	5.3	4.2
Met.#	2.1	1.8	0.9	2.7	2.3	2.2
Ile.	2.8	3.1	3.0	2.9	4.6	4.2
Leu.	5.4	6.4	6.7	5.8	7.2	4.8
Tyr.	3.4	1.1	1.3	2.1	4.2	2.8
Phe.	1.9	4.0	2.2	2.6	4.2	2.8
Lys.	10.3	11.5	7.5	9.5	8.4	4.2
His.	4.9	8.1	5.5	5.6	3.5	
Arg.	7.2	8.8	7.7	7.4	6.8	
Try.*	1.1	0.4	0.4	0.7	1.5	1.4
Amm.	2.8	3.1	4.2	2.8	1.4	
Total	102.7	109.9	103.7	113.7	107.6	

\# Performic acid oxidation separate determinations, by the methods of Schram et al. (1954) and Moore (1963).

* Method of Inglis and Leaver (1964).

a. Values from Rackis et al. (1971).

b. Food Agriculture Organization recommended pattern.

hydrochloric acid extract than those in the isolates P_tW and P_tA. The isolates P_tB and P_tS had most of the amino acids at higher levels than the isoelectric precipitated islates P_iB and P_iS. The isolated P_iW had 20 to 44% more lysine than any other isolate in the three stage extraction procedure.

The amino acid composition of the two preparations of the isolates P_i and P_t showed some differences in their distribution. The isolate P_tW had a higher threonine content than P_iW. The isolate P_tA had a higher sulfur containing amino acid together with glutamic acid than in the isolate $P_iA°$. The isolate P_iB had a higher cystine and methionine content and lower lysine and glutamic acid than in the isolate P_tB. In the isolate P_tS there is a higher concentration of tryptophan, tyrosine methionine and glutamic acid and lower levels of arginine than in the isolate P_iS.

Among the 8 isolates prepared, the hydrochloric acid extract contained higher levels of most of the essential amino acids. The isolate P_iB had higher glutamic acid, proline and cystine. It was observed that the isolates P_iS and P_tS had amino acid concentrations averaging those present in the isolates by three stage extraction, except for lower proline and higher histidine and arginine in isolate P_iS.

The values observed by Finlayson et al. (37) for the fraction AIVS for amino acids were lower except for higher amounts of glutamic acid and proline. But their isolate BI had a similar amino acid composition except for a

higher proline, aspartic acid and glutamic acid; and lower concentrations of lysine and tyrosine. These deviations in the amino acids is demished in the isolates P_t.

It is interesting to note that these isolates had a higher range of threonine (35 to 51%), glycine (25 to 35%), alanine (18 to 34%), lysine (14 to 32%), histidine (42 to 58%), and arginine (10 to 38%), and lower levels of aspartic acid (32 to 41%), glutamic acid (30 to 38%), phenylalanine (33 to 50%), and proline (12 to 43%) compared to those present in the soybean protein isolates (53). Only the isolates $P_i B$ had a 20% less lysine content than the soybean protein isolate.

Compared to casein (53) the isolates P_i showed higher amounts of threonine (8 to 37%), alanine (41 to 53%), cystine (61 to 86%), and arginine (39 to 58%). In addition the isolates $P_i W$ and $P_i S$ had higher amounts of lysine while the other fractions were lower than those present in casein. The isolates P_t were quite similar to casein with regard to the amino acid composition.

The amino acid content in rapeseed whey protein after isoelectric precipitation was higher than that of the respective isolates P_i. The same phenomenon was observed for soybean protein isolates (72). Compared to the whey from soybean protein, the whey from isolates $P_i W$ contained higher threonine, alanine and lysine and lower asparatic acid iso-leucine and phenylalanine. The whey from isolates $P_i A°$ was

higher in histidine and glutamic acid. The whey from isolate P_iS was high in glycine, glutamic acid and histidine and an exceptionally high concentration of cystine. The whey from $P_iA°$ indicated concentrations of basic amino acids higher than those in the other wheys.

The rapeseed protein concentrates prepared by Lo and Hill (53) had similar aminco acid composition to the concentrate CS except lower values by 30% threonine, 22% serine, 33% alanine, 21% cystine, 35% methione, 31% tyrosine, and 39% tryptophan and 12% more proline. Thus it appears that this single stage extraction endorses the good recovery of sulfur containing and other essential amino acids.

Compared to the soybean protein concentrates, the rapeseed protein concentrate CS contains higher amounts of phenylalanine and isoleucine by 30 and 25% respectively and 19 and 89% higher threonine and sulfur containing amino acid contents. Thus the high concentration of sulfur containing amino acid in rapeseed protein concentrate CS compared to the amount present in the soybean protein concentrate may be an advantage to the wide use of this preparation.

H. Ultracentrifugation

Ultracentrifugation pattern of the first water extract isolate prepared by isoelectric precipitation is given in Figure 18. There were three peaks observed under the experimental conditions used. The respective sedimentation coefficient of the three peaks were 5.4S, 3.0S and 0.9S.

Figure 18. Schlieren patterns of the first water extract

isolate P_iW. Concentration 1.0%; 0.1 M Tris-glycine

pH 8.5 containing 6 M urea and 0.1 M 2-mercaptoethanol.

The pictures were taken 25 mins (upper) and 60 mins

(lower) after reaching 59,000 r.p.m.
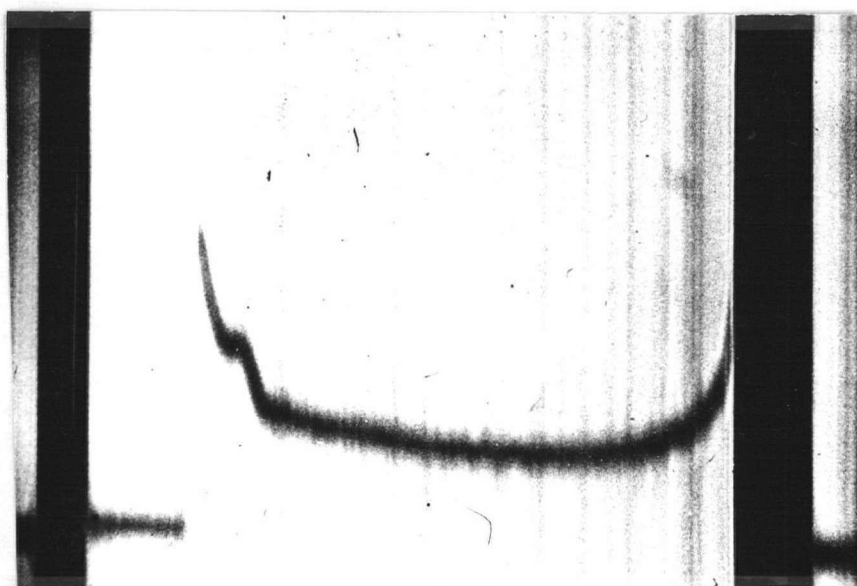
Figure 19. Schlieren pattern of the second hydrochloric
acid extract isolate $P_iA°$. Concentration 1.0%;
0.1 M Tris-glycine pH 8.5 containing 6 M urea and
0.1 M 2-mercaptoethanol. The picture was taken
20 mins after reaching 59,000 r.p.m.
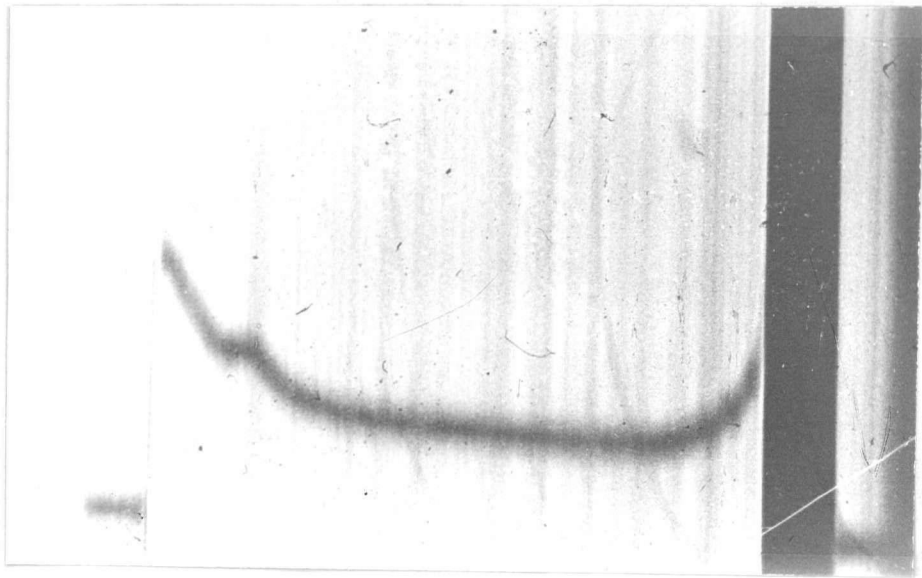
Figure 20. Schlieren pattern of the third sodium hydroxide

extract isolate $P_i B$. Concentration 1.0%; 0.1 M

Tris-glycine pH 8.5 containing 6 M urea and 0.1 M

2-mercaptoethanol. The picture was taken 65 mins

after reaching 59,000 r.p.m.

Figure 21. Schlieren pattern of the single stage sodium

hydroxide extract isolate $P_i S$. Concentration 1.0%

0.1 M Tris-glycine pH 8.5 containing 6 M urea and

0.1 M 2-mercaptoethanol. The picture was taken 60

mins after reaching 59,000 r.p.m.
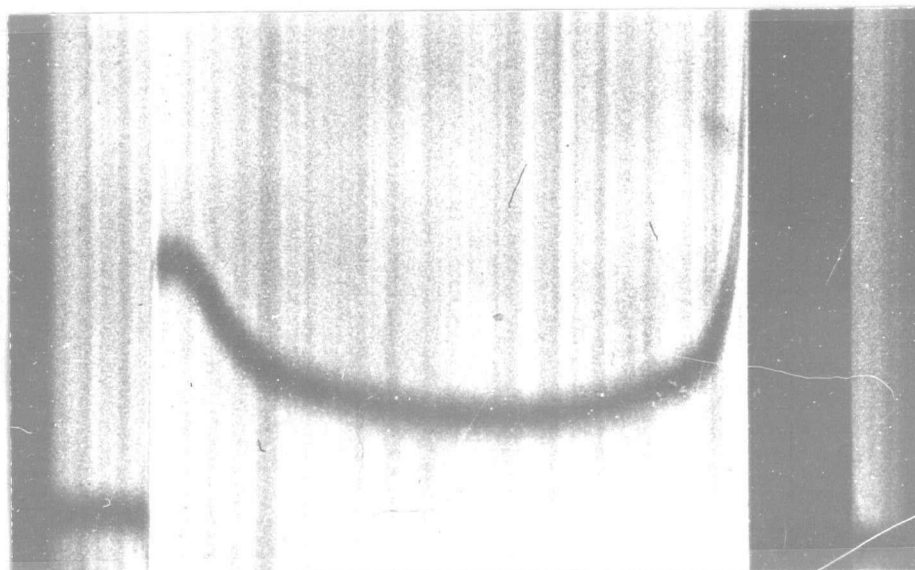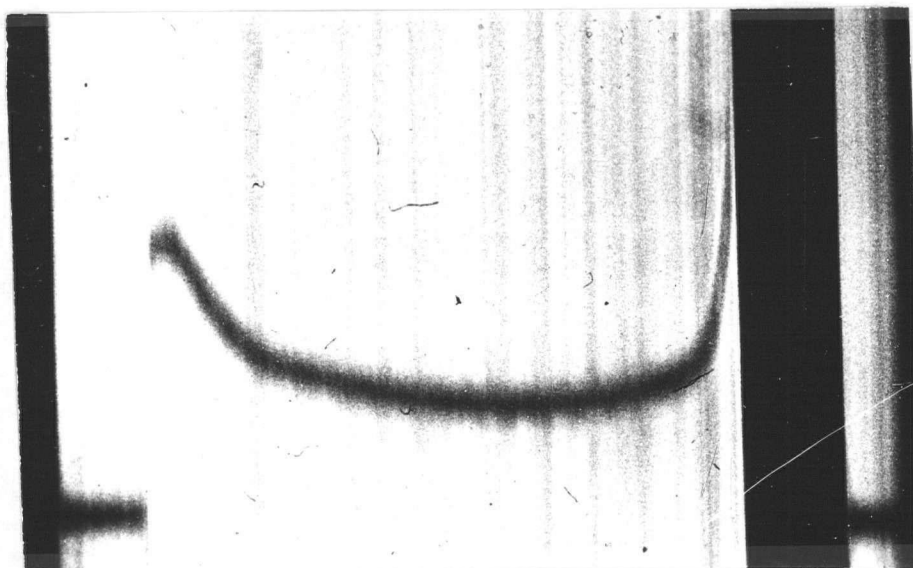
The ultracentrifugation pattern of the second hydrochloric acid extract isolate prepared by isoelectric precipitation is presented in Figure 19. A single peak was observed by this preparation. The sedimentation coefficient under the conditions described in this experiment was 1.9S.

The ultracentrifugation pattern of the third sodium hydroxide extract and single sodium hydroxide extract isolate prepared by isoelectric precipitation had sedimentation coefficients of 0.2S and 0.4S respectively. These patterns are shown in Figures 20 and 21. The third sodium hydroxide extract isolate had a large number of fast moving peaks which disappeared within the first 3 minutes.

I. Material balance of isolates

The recovery of proteins by isoelectric precipitation was low. This is evident in the high levels of nitrogen in the rapeseed whey obtained from different fractions indicating that much of the extracted protein was not precipitated. The nitrogen loss in the whey from trichloroacetic acid precipitation was less than what was observed for the isoelectric precipitation. This is given in Table VIII.

J. Loss of protein during washing procedure

A considerable loss of protein was observed during the washing process. The loss of protein by washing the trichloroacetic acid precipitated isolates P_t was greater than in the isoelectric precipitated isolates P_i. This loss of protein in washing is shown in Table IX.

TABLE VIII

PROTEIN MATERIAL BALANCE FROM THE PREPARATIONS
OF RAPESEED PROTEIN EXTRACTS AND ISOLATES
(as percentage of dry matter in original
flour).

	Extract	Whey P_i	Whey P_t
First water extract	5.5	1.6	0.4
Second acidic extract	5.8	3.2	0.5
Third basic extract	20.4	5.4	0.9
Total	33.7	10.2	1.8
First basic extract	26.8	4.6	1.5

TABLE IX

PROTEIN LOSSES FROM WASHING
THE ISOLATES WITH WATER AND
50% ALCOHOL (as percentage
of isolates).

	Water washing		Alcohol washing		Total washing	
	P_i	P_t	P_i	P_t	P_i	P_t
1st water extract	0.02	0.41	0.01	0.01	0.03	0.42
2nd acidic extract	0.02	1.09	0.02	0.38	0.04	1.47
3rd basic extract	0.11	].17	0.04	0.77	0.15	2.94
Total losses	0.15	3.67	0.07	1.16	0.22	4.83
1st basic extract	0.09	2.51	0.11	2.49	0.20	5.00

K. Yield of protein isolated

The yields of protein isolated are tabulated in Table X. With the isoelectric precipitation at 35°C the yields obtained were 10.8, 7.2 and 41.5% respectively for the three stage extraction and 61.2% for the single stage extraction. For the trichloroacetic acid precipitation the yields obtained were 14.2, 14.7 and 53.8% respectively for the three stage extraction and 70.3% for the single stage extraction.

L. Solubility measurements of the isolates
    and concentrates.

The results indicate that the solubility of the isolates P_iW and $P_iA°$ were approximately 59 and 49% respectively, while the sodium hydroxide extract isolates P_iB and P_iS had a lower value of 42.5%.

The concentrates had a higher solubility for the three stage extraction 77, 80 and 71% for the samples CW, CA and CB respectively. The single stage sodium hydroxide extract concentrate CS had a lower solubility of 58%.

These results are tabulated in Table XI.

TABLE X

THE YIELDS OF PROTEINS ISOLATED

	Water extract	Acid extract	Basic extract	Single basic extract
			- - - -%- - - -	
Isoelectric precipitation	10.8	7.2	41.5	61.2
Trichloroacetic acid precipitation	14.2	14.7	53.8	70.3

TABLE XI

SOLUBILITY MEASUREMENTS OF THE ISOLATES AND
CONCENTRATES EXPRESSED AS PERCENTAGES
OF PREPARATIONS

	Isolate P_i	Concentrate
First water extract	58.7	76.9
Second acid extract	48.5	80.3
Third basic extract	42.1	70.8
First basic extract	43.2	58.4

CHAPTER V

DISCUSSION

The extraction of rapeseed flour at high alkaline regions over pH 10 resulted in a yellowish green product, while around pH 10 the colour was creamy yellow. The darkening of colour at higher pH is due mainly to the greater extractability of pigments present in the flour. Thus by controlling the pH of the solution the colour can be kept lighter. The isolate obtained by extracting at pH over 10 showed some trailing as observed in the polyacrylamide gel electrophoresis. A greater recovery of protein can be achieved at higher pH regions, but the colour of the protein was not satisfactory.

As seen from Figure 5, after reacting the hydrochloric acid extract with oxalic acid, it was observed that at the optimum concentration of 0.05 M there was no further increase in the calcium removed. Thus oxalci acid was able to combine and precipitate salts in the extract up to the maximum amount of calcium was removed.

The nature of the curve shown in Figure 6 indicates that the protein content in the supernatant increased after reaching the optimum oxalic acid concentration (0.05 M) of the second hydrochloric acid extract. The increase after the initial decrease may be attributed to solubilization of calcium proteinate. After reaching the optimum oxalic acid concentration there was a sudden decrease in the protein content observed. This sudden decrease may be due to salting out of

some protein.

As shown in the Tables I and II, the isolates prepared by trichloroacetic acid precipitation P_t of the extracts showed higher values for moisture and protein while the isolates prepared by isoelectric precipitation P_i had higher values for carbohydrates, ash, phosphorus, sulfur and thiocyanates. The fat and crude fiber content was generally about the same for both preparations. These results would be e xpected because of the higher purity of the trichloroacetic acid precipitation P_t with greater protein content and lower concentrations of other compounds.

The concentrates, as expected, show a lower percentage of protein and higher levels of moisture, ash, calcium, phosphorus and crude fiber. The sulfur content ranges similarly to isolates prepared by isoelectric precipitation P_i. The most interesting feature of the concentrates was the lower isothiocyanate content compared to the two isolates.

The results for the second hydrochloric acid extract isolate after the treatment with 0.05 M oxalic acid showed that not only the ash content was reduced from 36.5 to 9%, but also the phosphorus content was decreased from 11.6 to 1.8%. This reduction of phosphorus on oxalic acid treatment may be due to complex calcium salts formed during precipitation.

The values for the protein isolates obtained were low compared to the published data for other vegetable protein isolates such as soybean, cotton seed and sunflower. This

may be due to the high ash and lipid content in the isolates.

The high carbohydrate content of the first water extract concentrate may be due to the easy solubility of the sugars during the extraction. The carbohydrate content of the isolates prepared from the water extract was low due to the precipitation of the proteins during their preparation. The whey therefore should have contained most of the contaminating carbohydrates.

Sulfur content was lower in the hydrochloric acid extract concentrate CA, than in the isolate P_iA. Whereas for the other extractions, the concentrates had a higher sulfur content than in their respective isolates. This loss of sulfur during precipitation into the whey may be due to incomplete precipitation of the sulfur containing protein fractions or peptides.

The high phosphorus content in the first water extract isolate P_iW may be due to the high solubility of the phytic compounds present in the rapeseed flour. Among the concentrates the second hydrochloric acid extract CA had an even higher phosphorus level caused by the solubilization of the compounds in the acid media.

As mentioned earlier the calcium content of the second hydrochloric acid extract isolate P_iA after oxalic acid treatment was reduced by approximately 50%. The reduction of calcium in the hydrochloric acid isolate P_iA on oxalic acid treatment is due to the ability of oxalic acid to combine

and precipitate salts in the extract. Generally the calcium content in the isolates and concentrates were high.

Generally the ash content of the isolates and concentrates were higher than those reported for other vegetable protein isolates. This high ash level in the preparations may be attributed to the amounts of calcium present in them. The ash content of the hydrochloric acid extract precipitated by trichloroacetic acid was 1.0%, while the isolate prepared after oxalic acid treatment was 2.0%. This could be due to the buffering action of oxalic acid which causes the co-precipitation of calcium salts with the protein during trichloroacetic acid precipitation.

Crude fiber content was fairly high in the isolates, especially in the second hydrochloric acid extract preparation P_iA. The high value may be due to the fine particles in the supernatant that tends to precipitate with the proteins during pH adjustments. The published data for soybean protein isolate and concentrate were 0.7 and 3.0% respectively.

From the result it was apparent that the isothiocyanate level of the water extract preparations were higher than that observed for any of the other preparations. This high isothiocyanate content indicated in the water extract is probably due to the higher extractability of this material in water at 4°C.

It is seen that with successive extractions in the three stage procedure the isothiocyanate content decreases. The isothiocyanate present in water extract isolates and

concentrates by this procedure were approximately 25% higher than that for the single stage sodium hydroxide extract, exept for the isolates prepared by isoelectric precipitation P_i where the single stage sodium hydroxide extract is higher by approximately 8%.

Slab gel electrophoresis of the first water and second hydrchloric acid extracts isolates P_i indicated separation of acidic and neutral proteins from basic protein as confirmed at pH 8.8. Thus with this simple three stage extraction procedure it is possible to separate the protein fractions containing important amino acids from rapeseed flour. These fractions could be used in food processing to improve the nutritional value.

Considerable amount of protein is being lost in the whey from isoelectric precipitation and it is necessary to find out methods of preventing this loss in the future. Work is currently being carried out in this laboratory by using sephadex column chromatography to determine which fraction contains the highest amount of sulfur containing amino acids.

Amino acid composition of the whey from the single stage sodium hydroxide extract isolate revealed an extremely high concentration of cystine. This loss of cystine must be prevented as much as possible. The phenomenon is similar to that observed by Rackis et al. (72) for soybean protein isolate whey.

Each protein fraction from the rapeseed flour shows distinct characteristics regarding the amino acid composition when compared to casein and soybean protein isolates. It could be inferred that rapeseed protein isolates if properly utilized after preparation, much of the present demands for high quality proteins could be overcome without encountering difficulties.

As observed from the amino acid composition of the single stage sodium hydroxide concentrate CS contains a higher sulfur content amino acid compared to the soybean protein concentrate. This situation may be of some benefit to the consumer as isolates tend to lose this essential amino acid during precipitation.

Ultracentrifugation patterns for the rapeseed protein isolaes from the first water, P_iW and second hydrochloric acid extract, P_iA indicated that large molecules have broken down into units of small molecular weights. This dissociation is due to the blocking of the sulfhydryl and amino group present in the isolate, to facilitate solubilization of the insoluble proteins. Similarly the results obtained for the third sodium hydroxide extract P_iB and the single stage sodium hydroxide extract isolate P_iS indicated very low sedimentation coefficients.

The results published by Bhatty et al. (14) showed sedimentation coefficients of 12S and 1.7S for their preparation BI. The results obtained in this study, 1.9S value for hydrochloric acid isolate P_iA, closely agree with their

sedimentation coefficient.

From the results obtained it is evident that the isolates from the three stage extraction procedure precipitated by trichloroacetic acid give yields of 14% more than from the single stage extraction procedure, while the yields for the isoelectric precipitation are approximately the same for t he two procedures. The low value for the isoelectric precipitation of the three stage extraction procedure should have resulted due to only partial precipitation of the proteins.

There is a great loss of protein during washing the isolates prepared by trichloroacetic acid precipitation, which could be due to the strong precipitating ability of trichloroacetic acid for small peptides which may be lost during the washing operations.

The solubility of the isolates in water is not very high for preparations. This insolubility may have been a result from the initial heat treatment of rapeseed meal to which it was subjected during the preparation of flour and during the elimination of toxic compounds. The protein could have been denatured during the adjustment of pH to their iso-electric points. All these may have played a role in the decreased solubility of the isolates. The solubility of the concentrates prepared from the three stage extraction procedure was reasonably good with values ranging from 70 to 80%, while the single stage sodium hydroxide extract concentrate was only 58%.

PART II

FUNCTIONAL PROPERTIES OF THE RAPESEED

PROTEIN FRACTIONS

CHAPTER VI

INTRODUCTION

The modern use of isolated proteins and protein concentrates for food is in an early stage of development. Progress will depend largely on technological and basic research applied to their production. At present the chief function is to improve the nutritional quality of the bread by supplementation of the protein in wheat flour.

Rapeseed protein isolates and concentrates may be useful nutritionally because of the high content of sulfur containing and basic amino acids. Therefore these preparations could become rich sources of supplementary material for foods deficient in them. Another important feature of rapeseed protein isolates is the absence of any beany flavour characteristic to soybean protein isolates. With methods of processing and techniques for eliminating glucosinolates, it is very likely that rapeseed meal will become a good source of protein that can be used like the conventional vegetable protein isolates and concentrates that are available easily today.

At this stage of our research we are hopeful of finding more useful applications in other areas of food processing, for instance, water holding capacity in meat or bakery products; contraol of fat absorption; viscosity characteristics; gel formation; texturability; feathering properties in coffee; aerating capacity in whipped toppings and frozen desserts.

CHAPTER VII

LITERATURE REVIEW

Rapeseed protein isolates and concentrates have not been so far fully utilized in any significant extent, for supplementation or fortification or enrichment of common food commodities such as bread, ice cream, spreads and meat products. Reference to recent literature indicates that soybean protein isolates have been successfully used to enrich the above products commercially (85). It was noted that when soybean protein isolates were used, there were certain improvements in some physical properties of certain foods. These properties include water binding, emulsifying, stablizing, thickening and film and dough forming ability (4, 42, and 48).

It is known that the addition of 3% soybean protein to albumen improved the whip, giving a higher whip volume in a shorter time. Recently Tsen and Hoover (92) reported improvements of nutritional value of bread. Bread being a basic food stuff the world over, presents a logical means of improving the protein intake in the diet of many hungry or malnourished people in developing countries. This enrichment or fortification must be done at levels which will significantly improve the nutritional value of bread without sacrificing other qualities of the finished product. Unfortunately, the addition of protein to wheat flour has changed some of the structural and functional properties of

the product such as flavour, colour texture, appearance, aroma and volume. It has been impossible to add any significant amounts of nonwheat nutrient additives to bread without losing desirable qualities.

With the use of isolated protein fractions as a basis for the formulation of duly acceptable, high protein food products at reasonably low costs have been developed (21).

There is a considerable need to improve the protein supply in order to overcome famine and disaster in underdeveloped areas of the world. The conventional means of improving protein supply is by increasing agricultural production and conversion of plant to animal protein can be properly and efficiently exploited. At present there is much interest in new sources of protein: fish from the sea, yeast grown on petroleum, chemical synthesis, improved photosynthesis and by plant cell culture (2).

There is no doubt that important advantages have been made during the past 15 years in utilizing vegetable protein sources in the fight against protein shortage (79). Probably the most important advantage made is that vegetable proteins properly processed and efficiently combined with one another can replace animal protein food in nutrition of man and, expand the availability of dietary protein, now so scarce (15). This has been possible because of accumulated knowledge of protein nutrition indicating that amino acid

pattern, not protein as such, is the most important factor in determining the nutritive value of the food protein (73).

Considering the significant inefficiency of converting vegetable protein into animal protein, it is perhaps quite logical that research workers in Food Science have given due thought to the direct use of vegetable proteins in human food. It is known that 1 pound of utilizable protein from beef costs $3.20, while that from soybean and wheat costs only 31 and 41 cents respectively (97).

It has been reported from India by Chandrasekhara et al.(17) that toned and double toned milk can be made by mixing reconstituted skim milk solids with cow milk. Miltone vegetable toned milk, is now aimed at replacing imported skim milk powder by indigenously available vegetable protein, mainly from peanuts. The deodourized protein solution is mixed with glucose, vitamin premix and standardized milk. The miltone thus obtained is homogenized and pasteurized and then cooled to 4°C and bottled.

Imitation milk has lost much of the popularity which it had in 1968. In addition to being inferior to cow's milk, the low protein level required to produce a cost advantageous product creates difficulties in achieving duplication of mouthfeel and body. Because of using protein derivates in imitation milk products, the preparation lacks the residual emulsifying power of nonfat milk solids, so the deficiency is made up by adding a higher level of mono- and

diglyerides. Imitation chocolate milk drink requires an additional quantity of kappa carrageenan to stabilize the cocoa fibers, because of the low protein levels of the drink. Phosphates or citrate salts are occasionally added to help in the colloidal solubilization of the protein. These salts are not necessary except when highly denatured protein is used with 0.1 to 0.2% of the salts (76).

Fortification of foods with synthetic methionine, lysine, threonine and tryptophan, which are commercially available at reasonable costs, can be done without much difficulty. However, there is a possibility that the amino acid thus supplemented is readily lost during cooking and food processing. Furthermore, the free amino acids may affect flavour and colour through degradation or interaction with other food components, for instance by Strecker degradation or amino-carbonyl reaction. Therefore the isolation of nutritionally well balanced protein must still be the primary importance in food processing.

Fijimaki et al. (39) have developed a method of rectifying amino acid imbalance in most vegetable proteins by digesting protein with a protein hydrolyzing enzyme and resynthesized a protein like substance (plastin) with the help of a proteinase after condensing the hydrolysate. They could even couple an amino acid ester such as methionine ethyl ester directly to plastin molecule instead of using methionine-containing protein hydrolysate. As a result

they were able to increase the sulfur containing amino acid
content by six to seven times.

Soybean protein supplementation of bread affords
a means of increasing the nutritive value with a better
balance of amino acids (35). Diser (27) has reviewed
literatures on soy flour and soy grits as protein supple-
mentation. Polloch and Geddes (69) reported work in which
1% unheated soy flour improves the bread, but higher levels
decrease the loaf volume.

It is well known that gluten protein complex is
a functional protein system which interacts with itself and
with other flour constituents to form dough that will
retain gas and produce bread of acceptable loaf volume.
Pence et al. (67) showed that crude albumen fraction iso-
lated from water solubles is responsible for the larger loaf
volume.

Harris et al. (45) illustrated that supplementation
of wheat flour with 5% soy flour was equivalent to supple-
mentation with 5% nonfat dry milk. Small amounts of polar
lipids from wheat flour substantially improve volume and
freshness retention of bread (70). Preliminary investiga-
tions by Daftary et al. (25) have indicated that the improve-
ment was mainly due to glycolipids. Pomeranz et al. (71)
showed that commercially available sucroesters counteract
the deleterious effects up to 16% soy bean flour on loaf
volume. Glycolipids in which the fatty acids are directly

attached to the sugar moiety as in sucroesters were as effective improvers as glycosylglycerides in restoring loaf volume of bread baked with shortening from petroleum ether defatted flour. Comparison of the effect of synthetic glycolipids indicated that both hydrophobic and hydrogen bonds are playing important roles in bread making. Possibly there is an optimal hydrophile - lipophile balance (64) at which single lipid or lipid mixtures increase loaf volume of bread.

Dairy products can entirely or partly be replaced by plant products in different ways. For example it is the use of modern technology to make from vegetable oils, margarine that is very much like butter. It is further possible to make milk, ice cream and cheese from milk or plant protein and vegetable oils. It could also be possible to replace, in due course, milk or animal protein with plant protein, and milk or animal fat with vegetable fat (4).

We cannot overlook the oriental technology for making food based on oil seed proteins. To give a few examples, soy milk, bean curd and fermented products have been in demand for many centuries.

Pearson et al. (66) found that sodium soy proteinate to be a poor emulsifier in the usual pH range of meat and suggested that it probably does not serve any major function in emulsifying fat when added to sausage products. Swift et al. (89) stated that the participation of water

soluble protein in emulsion, formation is dependent on the
addition of sodium chloride. They reported that the amount
of oil emulsified per milligram of salt soluble protein
increased as the total amount of protein in solution was
decreased.

Sheikh et al. (80) have used rapeseed protein con-
centrates for the preparation of enriched bread. The
dietary studies on bread containing skim milk powder and
vitamin mineral mixtrue together with rapeseed or other
protein concnetrates have shown that enriched bread had
high protein, calcium and iron than unsupplemented bread.
They showed that there was only little differences in
digestion of various breads.

With the development of new protein rich food,
certain principles must be met to ensure commercial
acceptability. There are several criteria enumerated by
Beher et al. (12) and Van Veen (94) concerning the develop-
ment, testing and introduction of protein rich new products
as follows: The basic material utilized must be available
locally and not fully exploited already. The product must
be economical and should have long storage life for trans-
portation. They must be free of any toxic or deleterious
influence and must have an agreeable taste and odour, as
well as other desirable physical characteristics.

CHAPTER VIII

EXPERIMENTAL MATERIALS AND PROCEDURES

1. Materials

All materials for this work were purchased from the local market. Wheat flour, Robin Hood all purpose, enriched flour produced by Robin Hood Multifood Ltd., Montreal.

Eggs were a gift from the Department of Poultry Science, U.B.C., freshly laid.

Corn oil and coconut oil.

Margarine, Dalewood brand, manufactured by Westminster Foods Ltd., North Burnaby, B.C.

Yeast, Fleischman active dry yeast, Standard Brands Ltd., Vancouver.

Instant skim milk powder, Seven Farms, Westfair Food Ltd., Vancouver.

Emulsifiers, Atmul 124 and Atmos 300 manufactured by Atlas Chemical Industries.

2. Procedure

A. Microloaves baking method (10 gram. flour)

Bread was prepared according to the method of Shogren et al. (81) with some modifications with 5% protein preparations substituted for flour. The brew was prepared by suspending 0.25 g yeast in 6.7 ml water at 38°C containing 0.6 g sucrose, 0.05 g malt extract, and 0.15 g salt for 15 min. in a 35°C water bath. To this was added 0.3 g margarine and 0.05 g emulsifier Atmul 124 (a mono- and diglyceride mixture) and 10 g flour containing 5% rapeseed protein isolate or concentrate together with 0.05 mg potassium bromate. The dough was formed by mixing with a spatula for 2.5 min, by hand and allowed to ferment for 105 min. The dough was maintained at about 35°C throughout this period. The controls were made without emulsifiers. The dough was then punched by rolling on a plexiglas plate and molded to shape. The mold dough was allowed to proof for 60 min. at 38°C in greased pans 5.2 x 2.3 cm at the bottom. The proofed loaves were baked at 210°C for 10 min. in an oven. The baked microloaves were removed immediately from the pans and cooled on a wire mesh tray.

One hour after baking the microloaves were weighed and the loaf volume was measured by dwarf rapeseed displacement.

In another series of experiments rapeseed protein isolates from 1 to 10% was used for the manufacture of

microloaves.

Also the effect of emulsifiers on soybean protein isolate on bread making was compared with rapeseed protein isolate. In this 3 to 5% soybean protein isolate was used.

B. Crust colour determination

The method described by Ehle and Jansen (35) was followed for the determination of crust colour. The micro-loaves 1 hr. after baking were sliced and dried for 24 hr. at 75°C. The outer one sixteenth of an inch of crust was scraped off from the slices and ground in a mortar to a fine particle size. Five hundred milligram of this (dry weight) crust was then extracted with 25 ml of 50% ethanol for 4 hr. by mechanically shaking in stoppered Erlenmeyer flasks. Ten milliliters of the extract was centrifuged at 15,000 x g for 15 min. and the absorbance of the clear supernatant solution determined at 460 nm. The measurements are averages from duplicate extractions.

C. Determination of the emulsifying capacity

The method of Swift et al. (89) was followed for the determination of the emulsifying capacity of the protein isolates and concentrates from rapeseed flour. Samples of the isolates and concentrates containing 100 mg in 30 ml 1 N sodium chloride were heated to 80°C for 5 min. and blended in an omni mixer for 2 min. at ca. 1000 r.p.m. (See Plate III).

To this was added 20 ml corn oil and begin high speed cutting and mixing for 30 sec. at ca. 13,000 r.p.m. Addition of oil was continued at the rate of 0.8 ml per sec. till the break point was reached. The emulsion formed persisted, and finally collapsed, the transition being marked by gradual increase, followed by a sudden decrease in viscosity. The addition of oil was immediately terminated on observation of abrupt transition. The total oil added was noted. The emulsifying capacity is reported as the ml oil per 100 mg protein isolate or concentrate.

D. Stability tests for emulsions

Stability of the emulsions was determined by the method of Pearson et al. (66). For this determination the corn oil was added at the same rate as for the emulsifying capacity determination. The optimum level of oil obtained from the above experiment was added to the protein solution containing 100 mg in 30 ml of 1 N sodium chloride and blend at 13,000 r.p.m. for 2 min. The emulsions formed were transferred to 15 ml conical graduated centrifuge tubes to the 12 ml mark. The tubes were allowed to stand till the two phases were separated and the time recorded.

E. Determination of whippability

Whip tests were performed as described by Garibaldi et al. (40). Sixty gram of fresh egg albumen at 25°C was whipped at speed 10 in a Sunbeam mixmaster for 40 sec. (See

PLATE III

EMULSIFICATION EQUIPMENT - OMNI MIXER
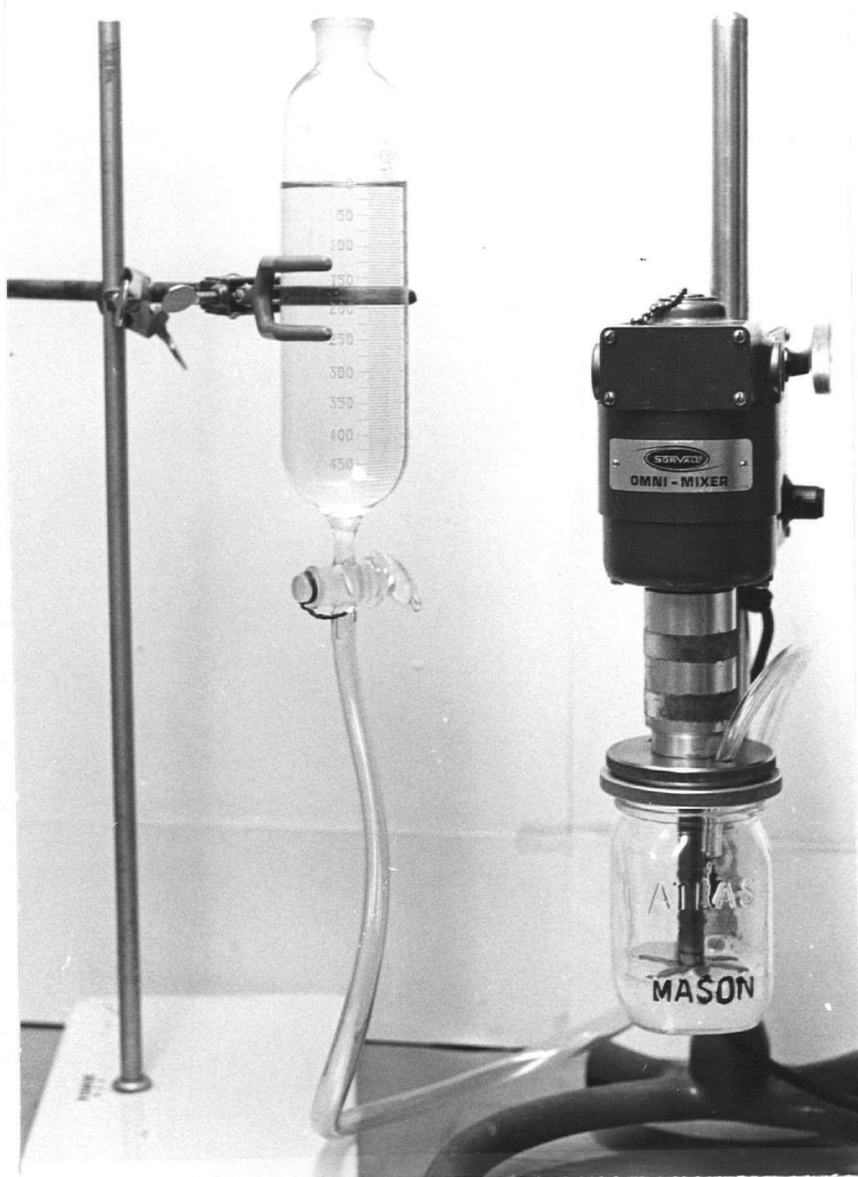
Plate IV). This was continued after changing speed to 6 with 0.25 rapeseed protein isolates or concentrates containing 46 g sucrose by adding in 3 equal portions with 4 sec. whipping after each addition. Controls were carried in the same way without either protein isolates or concentrates incorporated into the sucrose.

The specific gravity was calculated from the weight of the meringue in a funnel of known volume sitting on a 10 ml measuring cylinder. The reciprocal of the specific gravity gives the specific volume which is the expansion of the whip. The drip was also recorded for 1.5 hr. at 30 min. intervals.

F. Manufacture of spreads

Blends of 50 g of margarine, corn oil and or coconut oil were used in these experiments. The solid fats were warmed to room temperature 25°C and 0.5 g isolates or concentrates from the single stage sodium hydroxide extraction were blended for 30 sec. and heated to 80°C for 5 min. This was then cooled to room temperature and mixed at ca. 13,000 r.p.m. for 45 sec. and then continued at ca. 1,000 r.p.m. for 60 sec. The blends were placed in paper cups and were frozen with constant agitation in an ice and salt bath. These were stored at 4°C for periods of time and the consistency of the product noted.

PLATE IV

EQUIPMENT FOR WHIP MEASUREMENT - SUNBEAM MIXMASTER

Composition of blends:

No. 1   Margarine

No. 2   Margarine : corn oil : coconut oil (2:0.5:0.5 by wt)

No. 3   Margarine : corn oil (1:3 by wt)

No. 4   Margarine : coconut oil (1:3 by wt)

No. 5   Margarine : corn oil (1:1 by wt).

G.   Enriched imitation ice cream and ice milk

These milk products were made by blending 0.5 g
isolates with 12 g skim milk powder and dissolving in 90
ml water and heating to 80°C for 5 min. This was blended
for 30 sec. at ca. 1,000 r.p.m. in an omni mixer and cooled
to room temperature. Margarine, sucrose, span 20 as
emulsifier, gelatin as stabilizer, and vanilla or corn syrup
were added as indicated in Table XII. The mixture was blended
again for 30 sec. at 13,000 r.p.m. and at 1,000 r.p.m. for
60 sec. The emulsions formed were than pasteurized for
30 sec. by heating to 80°C. After pasteurization the
products were cooled and frozen by surrounding in a bath of
ice and salt, with constant agitation.

TABLE XII

COMPOSITION OF IMITATION ICE CREAM, CHOCOLATE
ICE CREAM, AND ICE MILK. THE BASIC INGREDIENTS
CONTAIN SKIM MILK POWDER AND PROTEIN ISOLATES
FROM RAPESEED.

Composition	Ice Cream	Chocolate drink	Ice milk
Margarine (g)	10.0	10.0	4.0
Sucrose (g)	15.0	12.0	15.0
Span 20 (g)	0.1	0.1	0.1
Gelatin (g)	0.3	0.3	0.3
Vanilla (drops)	1.0	-	-
Cocoa syrup (g)	-	15.0	-

CHAPTER IX

RESULTS

A. Microloaves of bread

With the preliminary experiments on the prepara-
tion of bread containing no emulsifiers the loaf volume was
decreased by approximately 10 to 15% when 5% rapeseed protein
isolates were substituted for flour. With the concentrates at
5% there was a 20% decrease in loaf volume. By using
emulsifiers the loaf volumes were restored for isolates.
The concentrates did not show much improvement in the loaf
volume with emulsifiers. The best emulsifier was Atmul 124
which increased the loaf volume of the bread prepared.

As seen in Table XIII, the bread prepared from
5% rapeseed protein isolates containing 0.5% emulsifier
Atmul 124, showed some improvement in loaf volume for the
first water and second hydrochloric acid extractions. These
two preparations had loaf volumes higher by approximately
10 and 14% respectively over the control. The isolates
from sodium hydroxide extracts showed no significant change
in the loaf volume. All results were obtained from dupli-
cate experiments. When the results differed greatly
additional experiments were carried out. The bread prepared
in this experiment is illustrated in Figures 22 and 23.

The bread prepared from flour containing 5% con-
centrates with 0.5% Atmul 124 showed a decrease of
approximately 8% except for the concentrate prepared from

TABLE XIII - a

THE LOAF VOLUMES OF BREAD AND THE COLOUR OF BREAD

CRUST CONTAINING 5% RAPESEED PROTEIN ISOLATES

	Volume loaf (ml)	Absorbance 460 nm
P_iW	56^b	0.04
P_iA	58^a	0.04
P_iB	50^c	0.07
P_iS	50^c	0.04
Control	51^c	0.025

'a', 'b' and 'c' are three groups significantly different
as shown by Duncan New Multiple Range Test. (Appendix
Table I).

TABLE XIII - b

THE LOAF VOLUMES OF BREAD AND THE COLOUR OF BREAD

CRUST CONTAINING 5% RAPESEED PROTEIN CONCENTRATES

	Volume load (ml)	Absorbance 460 nm
CW	48^c	0.03
CA	47^c	0.035
CBQ	57^a	0.06
CS	47^c	0.04
Control	51^b	0.025

'a', 'b' and 'c' are three groups significantly different
as shown by Duncan New Multiple Range Test. (Appendix
Table II).

Figure 22. Microloaves (10 grams) baked from rapeseed

protein isolates. From left to right, bread

containing the following:

W - first water extract isolate P_iW

A - second hydrochloric acid extract isolate P_iA

B - third sodium hydroxide extract isolate P_iB

S - single stage NaOH extract isolate P_iS

C - the control.

W   A   B   S   C
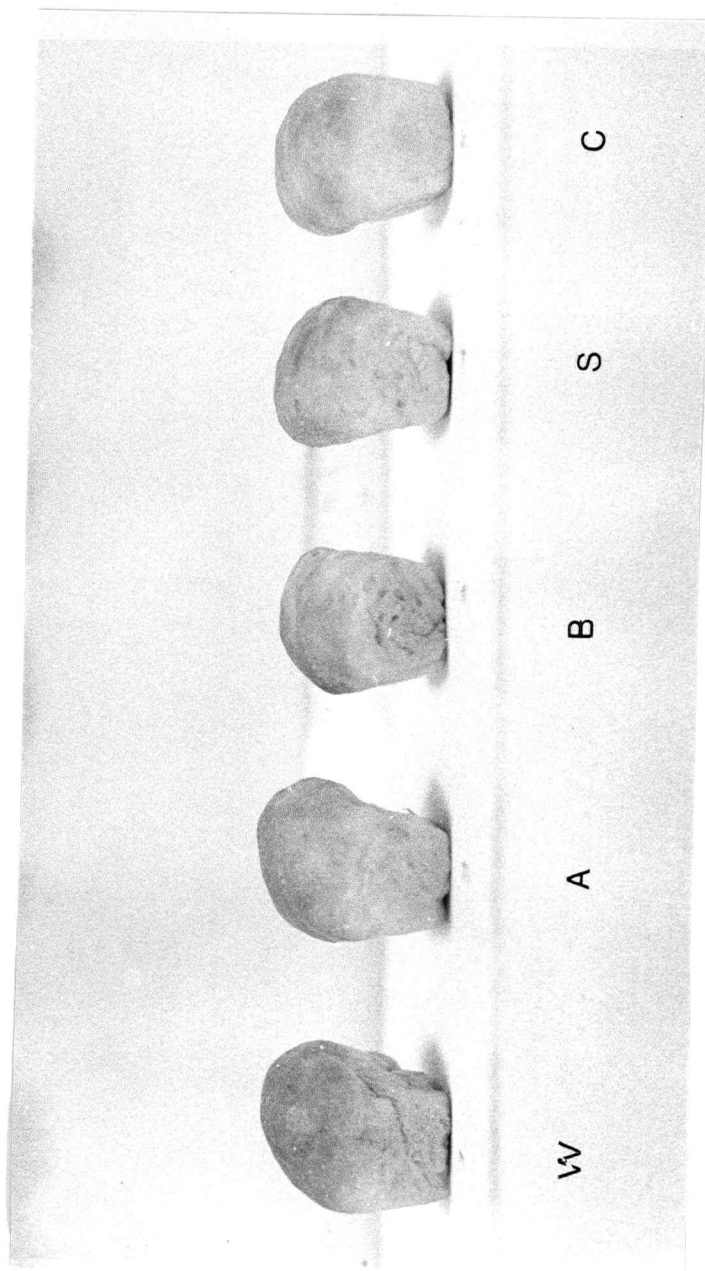
Figure 23. Microloaves (10 grams) baked from rapeseed

protein isolates. From left to right, cut loaves

of bread containing the following:

W - first water extract isolate P_iW

A - second hydrochloric acid extract isolate P_iA

B - third sodium hydroxide extract isolate P_iB

S - single stage NaOH extract isolate P_iS

C - the control.

W   A   B   S   C
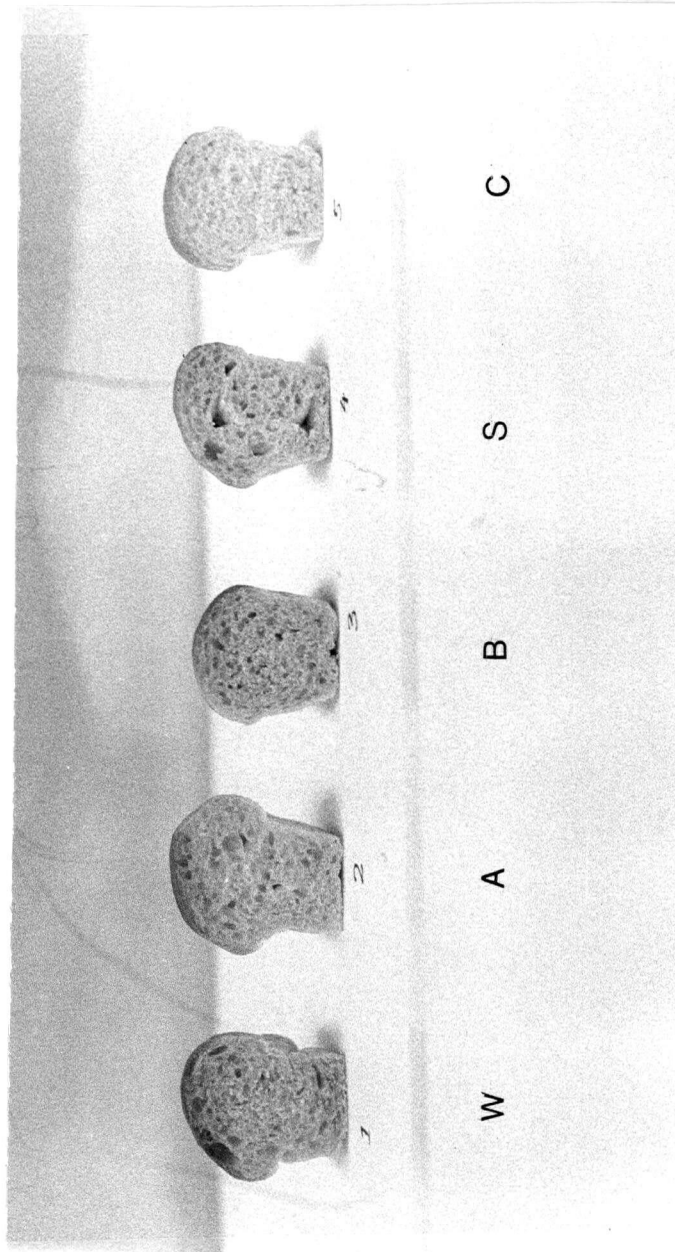
Figure 24. Microloaves (10 grams) baked from rapeseed
protein concentrates. From left to right, bread
containing the following:

W - first water extract concentrate CW

A - second hydrochloric acid extract concentrate CA

B - third sodium hydroxide extract concentrate CB

S - single stage NaOH extract concentrate CS

C - the control.

W   A   B   S   C
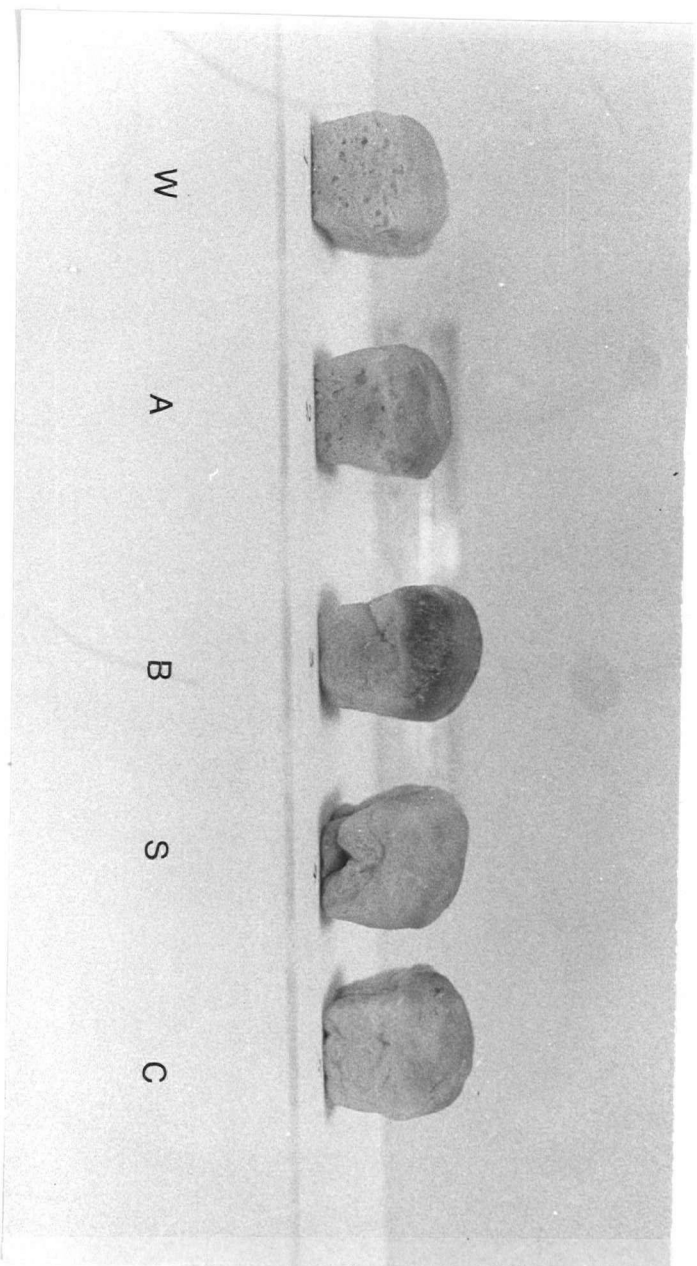
Figure 25. Microloaves (10 grams) baked from rapeseed

protein concentrates. From left to right, the cut

loaves bread containing the following:

W - first water extract concentrate CW

A - second hydrochloric acid extract concentrate CA

B - third sodium hydroxide extract concentrate CB

S - single stage NaOH extract concentrate CS

C - the control.

W   A   B   S   C
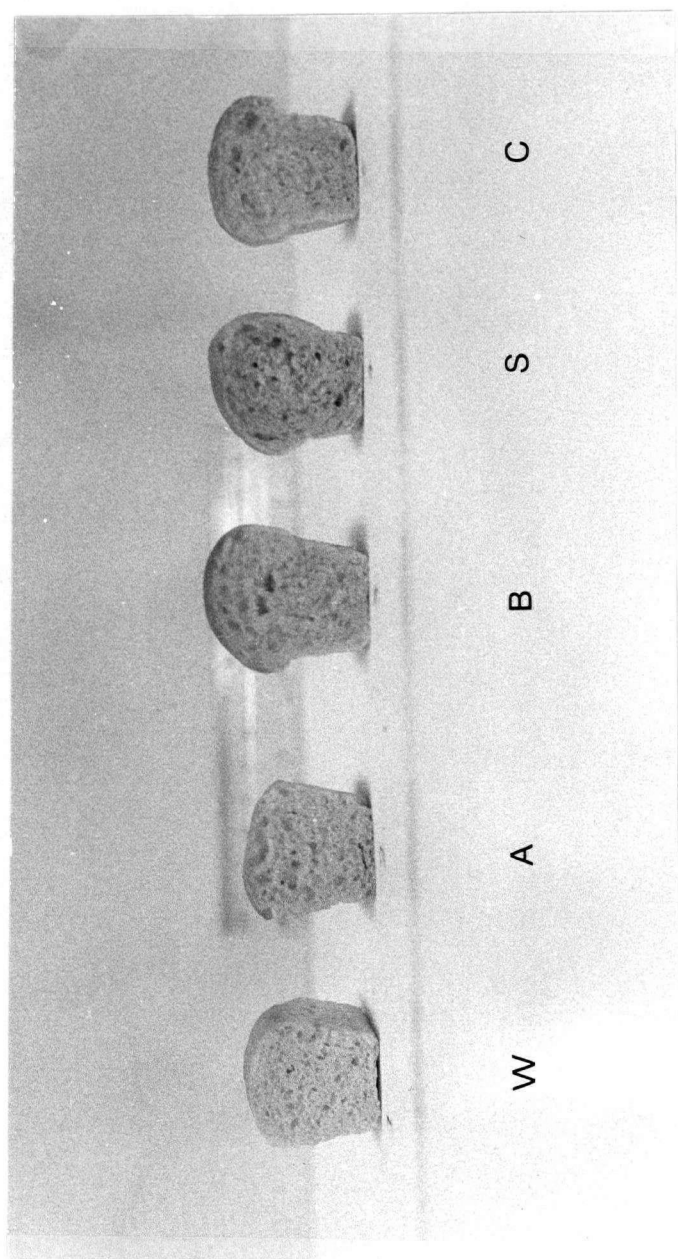
Figure 26. Microloaves (10 grams) baked from the single

stage sodium hydroxide extract isolate P_iS from

rapeseed flour. From left to right, bread containing

1, 2, 4, 6, 8 and 10 percent protein isolate, and

C - the control.

1 2 4 6 8 10 C
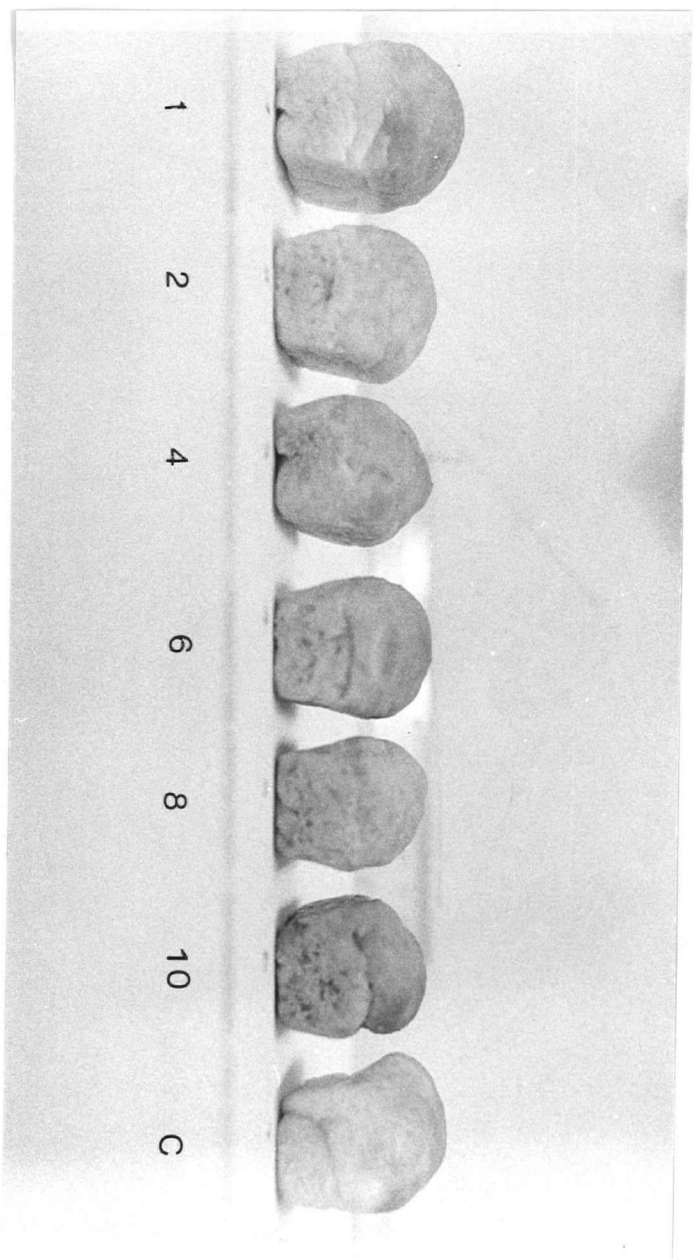
Figure 27. Microloaves (10 grams) baked from the single

stage sodium hydroxide extract isolate P_iS from

rapeseed flour. From left to right, cut loaves of

bread containing 1, 2, 4, 6, 8 and 10 percent

protein and C - the control.
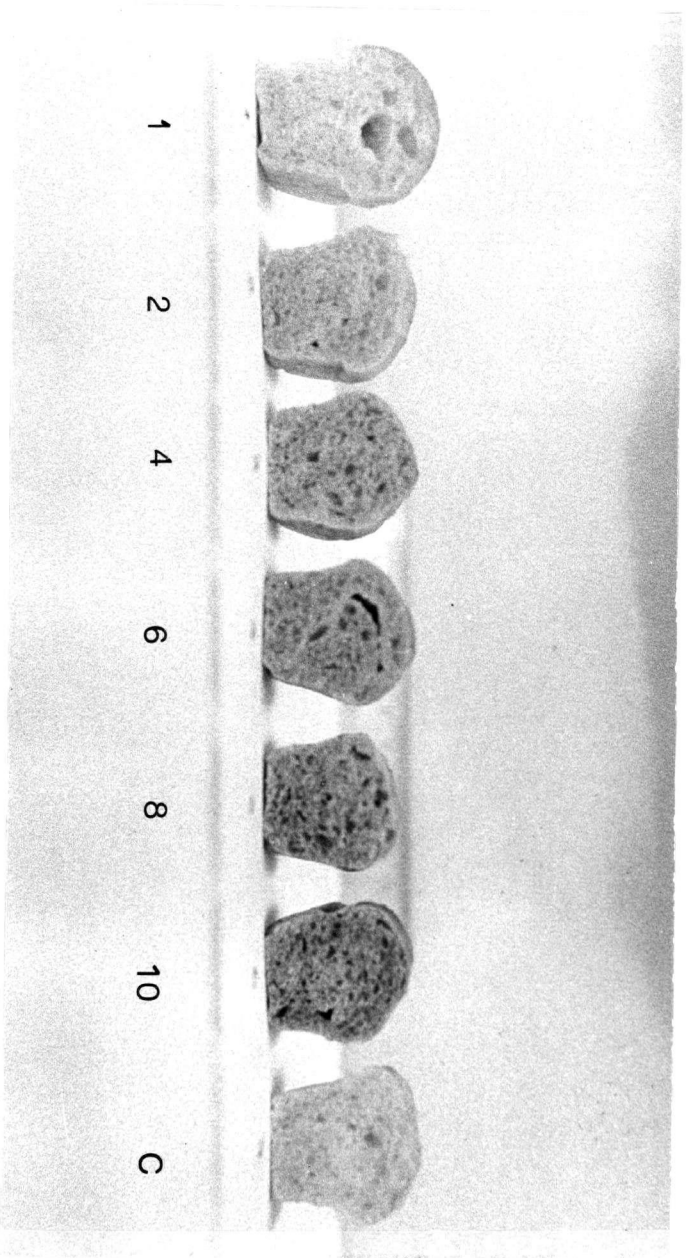
Figure 28. Microloaves (10 grams) baked from soybean

protein isolate containing 0.5% emulsifiers.

The composition of the bread is given below:

	Emulsifier	Protein isolate
Bread No.1	-	-
2	Atmul 124	3% soybean protein
3	Atmos 300	3% soybean protein
4	Atmul 124	5% soybean protein
5	Atmos 300	5% soybean protein
6	Atmul 124	5% rapeseed protein

1   2   3   4   5   6
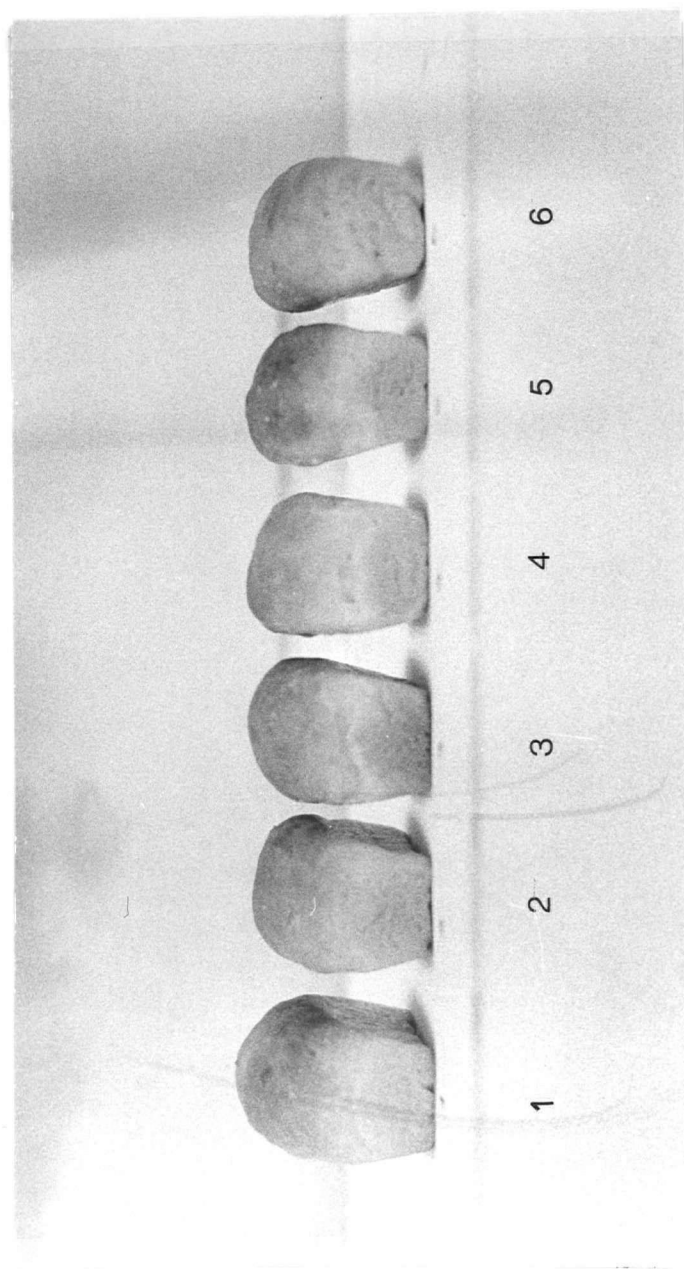
Figure 29. Microloaves (10 gram) baked from soybean

protein isolates containing 0.5% emulsifiers. The

composition of the bread is given below:

	Emulsifier	Protein isolate concentration
Bread No.1	−	−
2	Atmul 124	3% soybean protein
3	Atmos 300	3% soybean protein
4	Atmul 124	5% soybean protein
5	Atmos 300	5% soybean protein
6	Atmul 124	5% rapeseed protein
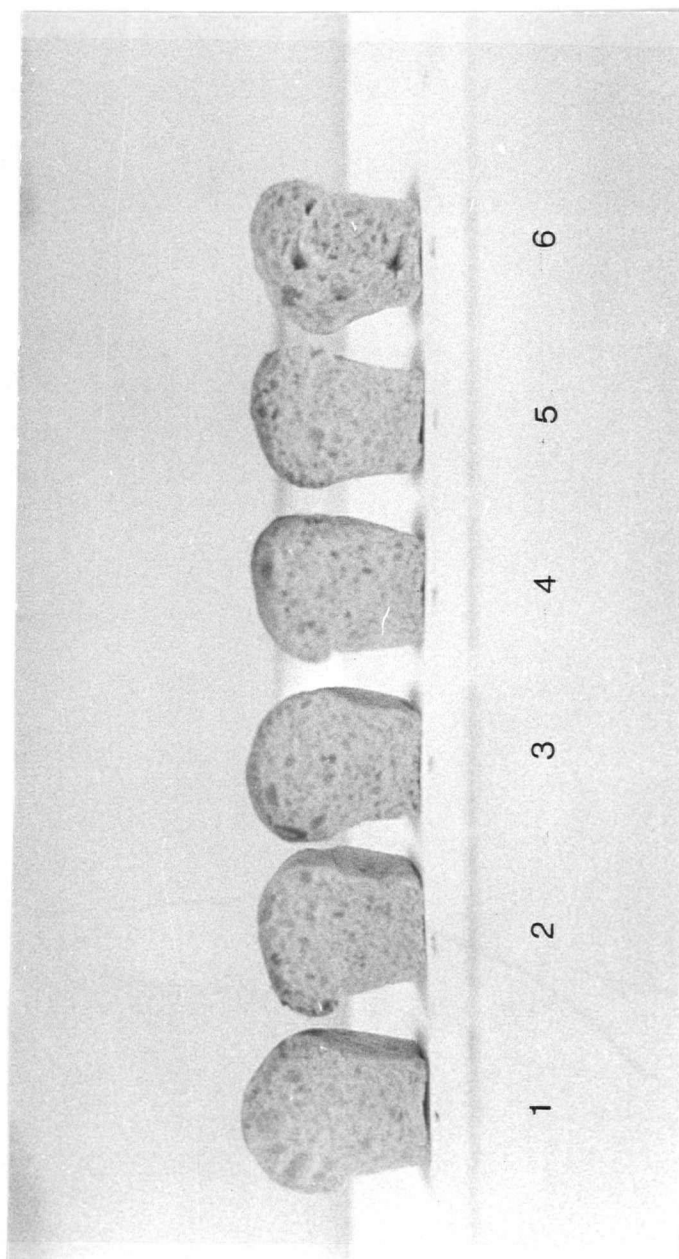
the third sodium hydroxide extract which had an increase of 12% over that of the control. These results are illustrated in Figures 24 and 25.

The effect of rapeseed protein isolate concentration on loaf volume and crumb colour is shown in Figures 26 and 27. The bread containing 1% rapeseed protein isolate from the single stage sodium hydroxide extract had a bigger loaf volume than any of the other bread prepared.

Figures 28 and 29 illustrate the effect of emulsifiers on protein from soybean on the loaf volume. The results indicated that Atmul 124 at 0.5% was slightly better than the emulsifier Atmos 300 at 5% soybean protein isolate. The 5% protein isolate had a slightly higher loaf volume than the bread containing 3% protein isolate.

The crust colour for the isolates and concentrates containing bread, except for the third sodium hydroxide extract preparation was a little higher than that observed for the control. The value obtained for the third sodium hydroxide extract isolate and concentrate was three times greater than that of the control. These results are tabulated in Table XIII.

B. Emulsifying capacity

From the results shown in Table XIV it is evident that the isolate from the first water extract has a higher emulsifying capacity than the other isoaltes by approximately 28%. This experiment suggests that the water extract

TABLE XIV

THE EMULSIFYING CAPACITY OF THE RAPESEED
PROTEIN ISOLATES AND CONCENTRATES, EXPRESSED
AS MILLILITERS CORN OIL PER 100 MILLIGRAM
PROTEIN ISOLATES OR CONCENTRATE AND THE
EMULSION STABILITY IN MINUTES.

	Volume of oil (ml)	Stability (min)
P_iW	45.0	10
$P_iA°$	36.1	200
P_iB	32.3	8
P_iS	37.2	8
Control	-	3
CW	33.1	7
CA	27.4	200
CB	25.2	7
CS	29.3	6

isolate is far superior to the rest with an emulsifying

capacity of 45 ml corn oil per 100 mg protein isolate. The

lowest value was with the third sodium hydroxide isolate

with 32 ml per 100 mg protein.

The concentrates showed a similar trend but with

a lower emulsifying capacity of approximately 25% than for

the isolates. Here again the weakest was the third sodium

hydroxide extract concentrate.

C. Emulsion stability

The stability of the emulsions formed was not as

expected, except for isolate and concentrate from the

second hydrochloric acid extract. The time taken for the

emulsions to break was under 10 min. for most while the

control was only 3 min. The isolate and concentrate from

the hydrochloric acid extract took over 200 min. to show any

signs of separation.

D. Whippability

(a) Expansion

The ability of the isolates to whip the egg white

shows that the hydrochloric acid extract was capable of

increasing the specific volume by 10% while the other iso-

lates decreased the expansion ability by approximately 16%.

These results are tabulated in Table XV.

When the concentrates were used for the whip

determination, the specific volume decreased in all samples,

with the water extract having a greater loss of 19% and the
hydrochloric acid and single sodium hydoxide extracts only
2%.

(b) Drip

As seen from Table XV the drip from the meringue
was negligible for all isolates and concentrates prepared
from the three stage extraction procedure during the first
30 min. The single stage sodium hydroxide extract prepara-
tions during this period showed a drip of 1.0 ml. With time
up to 90 min. all preparations showed 1.0 to 3.5 ml drip,
except for the water extract isolate, compared to 4.5 ml
drip in the control. The drip obtained from the concen-
trates was higher than that noted from the isolates in this
time period except for the concentrates from the third
sodium hydroxide extract.

E. The spreads from isolates

From preliminary experiments conducted the
isolates and concentrates of rapeseed protein was found to
be conveniently incorporated into butter like products so
that these could be used as spreads with high protein con-
tents. Among the preparations that were stored under
refrigerated conditions at 4°C, the sample No. 5 was soft
after 5 months, while the sample No. 3 was firm, and the
rest of the samples were hard like margarine. Thus it is
possible by proper blending of margarine, corn oil and or

TABLE XV.

WHIP TEST OF RAPESEED PROTEIN ISOLATES AND
CONCENTRATES, THE SPECIFIC VOLUME AND DRIP
OF MERINGUE WITH TIME.

	Specific volume	Drip of meringue (ml)		
		0.5 hr	1.0 hr	1.5 hr
P_iW	2.96	-	-	-
$P_iA°$	,3.74	-	0.6	1.0
P_iB	2.81	0.3	1.0	1.8
P_iS	2.88	1.0	1.8	2.7
CW	2.76	-	1.5	2.6
CA	3.33	0.2	2.1	3.5
CB	3.18	-	1.0	1.6
CS	3.31	1.0	2.0	3.2
Control	3.40	1.0	2.1	4.5

coconut oil with isolates and concentrates to prepare easily
spread emulsions that can be stored under refrigerated con-
ditions for long periods of time.

F.   Enriched imitation ice cream and ice milk

Enriched imitation milk products could be manufac-
tured at 1 to 2% isolates without imparting any undesirable
flavours into the product.   When the isolates were used at
5% level there was a detectable off-flavour from the
isolates, into the product.

CHAPTER X

DISCUSSION

As seen from the results obtained from the manu-
facture of bread containing the rapeseed protein isolates P_i,
from the first water and second hydrochloric acid extractions,
the loaf volume was increased by 10 to 14%. Thus it is
evident that the water soluble fractions, specially the
albumins are responsible for the loaf volume increase. This
is similar to what was observed by Pence et al. (67). The
large loaf volume obtained for the hydrochloric acid extract
isolate may be due to the basic nature of the protein
fraction.

The most intriguing observation was made with the
bread prepared with the concentrate from the third sodium
hydroxide extract, which was the only sample that was able
to increase the loaf volume of the bread. This increase of
the volume may be attributed to the higher fat content which
may have a large percentage of glycolipids and phospholipids.

It was observed from Figures 26 and 27, that with
the increase in concentration of rapeseed protein isolate
from the single sodium hydroxide, over 6% color of the bread
tends to darken to produce a brownish crumb. It is also
observed that with an increase in protein concentration there
is a gradual decrease in loaf volume. With 1% rapeseed
protein isolate the bigger loaf volume was obtained, than
others. The darkening of the crumb may be due to the

browning reactions taking place between the amino acids and sugars in the bread. Thus with an increase in the isolate concentration a darker crumb is produced causing a decrease in the nutritional value.

Crust colour results showed that the third sodium hydroxide extract isolate and concentrate had higher absorbance indicating the presence of higher glycolipids contents in this preparation. It is possible that browning reaction is enhanced by the type of carbohydrate and amino acids present or caramelization reaction taking place to produce a darker colour in the bread crust.

The high emulsifying capacity of the first water extract isolate could be due to the presence of phospholipids or glycolipids which enhance the emulsifying capacity. The water extract isolate P_iW had a greater stability than others except the second hydrochloric acid extract isolate which was extremely stable. The high emulsifying capacity of the water extract isolate could be due to the greater solubility of this compared to the others. The protein solution forms a protective coating around the oil droplets to give emulsion stability. The hydrochloric acid isolate which is extremely stable may be attributed to the same effect.

The whip test indicates there is a kind of difference among the isolates. The hydrochloric acid isolate P_iA caused a 10% increase in specific volume

compared to the others. This could be due partly to the basic nature of the protein fractions present in the isolate.

The concentrates were unable to improve the specific volume of the meringue. Among them the second hydrochloric acid and single sodium hydroxide extract concentrates decreased the specific volume by approximately 2%, while the water extract had a 19% decrease.

The drip in meringue could be decreased considerably by using the water extract isolate and by 78% with hydrochloric acid isolate. The third sodium hydroxide extract concentrate was able to decrease the drip by 65%. This behaviour of the third sodium hydroxide extract concentrate is due, as expalined earlier, to the composition of the preparation.

CHAPTER XI

GENERAL CONCLUSION

From the results observed from this investigation it w as concluded that by using the three stage extraction procedure, protein fractions rich in certain essential amino acids such as lysine and s ulfur-containing amino acids were separated without the use o f elegant chromatographic methods. A high ash content which is mainly due to the presence of large amounts of calcium is also an advantage, as this element is nutritionally important.

These individual fractions, especially the water and hydrochloric acid isolates, behave differently when applied to food processing such as bread manufacture, emulsion formation and whipping ability of egg albumen. The intriguing results from the third sodium hydroxide concentrate should be further investigated in order to utilize this fraction in the most economical way in the food processing industry.

From the results obtained so far, different protein isolates seem to be functioning better than others for special purposes in different food products. At this stage of this study it is hopeful that more useful applications in other areas of food processing, such as meat or bakery products, can be accomplished in the future.

APPENDIX

TABLE I

ANALYSIS OF VARIANCE OF LOAF VOLUME

WITH RAPESEED PROTEIN ISOLATES

Source of variation	Degree of freedom	Mean square	F-ratio
Replicates	5	24.32	
Isolates	4	80.60	175.23**
Error	20	0.46	-
Total	29		

** Significant at probability 0.01

Duncan New Multiple Range Test:

Loaf volumes of the bread prepared from the isolate P_iA is significantly different from that of P_iW, and these in turn differ significantly from the rest of the isolates and the control.

Isolate treatment	Control	P_iW	P_iA	P_iB	P_iS
Mean value	51.33	56.12	58.00	50.33	50.17

TABLE II

ANALYSIS OF VARIANCE OF LOAF VOLUME

WITH RAPESEED PROTEIN CONCENTRATES

Source of variation	Degree of freedom	Mean square	F-Ratio
Replicates	5	0.38	
Concentrates	4	115.98	85.91**
Error	20	1.35	
Total	29		

** Significant at probability 0.01

Duncan New Multiple Range Test:

      Loaf volumes of bread prepared from the rapeseed protein concentrate CB is significantly different from that of the control and these in turn are different significantly from the rest of the concentrates, which are similar to one another.

Concentrate treatment	Control	CW	CA	CB	CS
Mean value	51.33	47.67	46.67	57.00	46.83

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