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SOYA PROTEIN ISOLATE PRODUCTION

BY VARIOUS METHODS

BY

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I declare that the contents of this thesis, except where otherwise indicated in the text, are my own original work.

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ABSTRACT

SOYA PROTEIN ISOLATE PRODUCTION BY VARIOUS METHODS

The concentrated protein fractions of soyabeans, known as soya protein isolate, was produced by three different methods from the same raw material namely defatted soya flakes. Extraction of the soluble fraction of the raw material is common to all three methods. A study was therefore undertaken to optimise the extraction process conditions in terms of time, temperature, pH, extraction time, extraction volume and raw material particle size, thereby maximising yields of soluble material.

The three different methods, namely isoelectric precipitation, ultrafiltration and swollen gel technology were then used to separate the soluble and non-soluble protein fractions. Both the isoelectric and ultrafiltration methods gave good yields of finished product, with the ultrafiltration process giving the better overall yield, but the swollen gel method gave disappointing results and was not feasible in practice.

Functional properties of the products from the isoelectric and ultrafiltration methods were compared and found to be broadly similar although different in certain respects from those of commercial soya isolates.

Levels of the anti-nutritional factors trypsin inhibitor and phytate in products from the three processes were determined and the substantial differences observed in trypsin inhibitor levels

were further investigated. Determination of lysinoalanine levels was also attempted but the results obtained were unsatisfactory. Amino acid composition and polyacrylamide gel electrophoresis were used to compare the chemical composition of products from the three processes. The comparative economics of the isoelectric and ultrafiltration processes for large scale production of soya protein isolates were evaluated, taking into account the comparative efficiencies of the two processes as determined during the study. It was established that, while the isoelectric process initially appears more economical, it may be possible to modify the ultrafiltration process in such a manner as to make it more economical than the isoelectric process. Overall figures however indicate that the manufacture of soya protein isolate in South Africa is not currently a viable economic proposition, due to high raw material costs.

1. INTRODUCTION

The value of vegetable protein and oil seed protein in particular is widely acknowledged and has been extensively documented. (Altschul 1958; Gould 1966; Smith & Circle 1977; Norton 1978; Hudson 1982). They constitute the major source of protein for a substantial majority of the world's population and, when correctly processed, possess highly acceptable nutritional properties. While consumption of animal protein may be desirable from the point of view of variety of diet and in order to provide certain other nutrients not found in sources of vegetable proteins, a correct choice of vegetable proteins can more than adequately fill both human and animal protein requirements.

A further advantage of vegetable protein is its comparatively low cost of production and greater efficiency of resource usage in relation to animal protein - a vital factor when considering the nutritional requirements of less affluent societies and satisfying global food requirements.

Protein contents of the various sources of vegetable protein fall broadly into two categories (Norton 1978). Cereals have approximate protein contents varying from 8% (rice) to 13% (oats), whereas approximate legume and oilseed protein contents vary from 12% (sunflower seeds) to 38% (soya beans).

Considerable effort has gone into the extraction and concentration of the protein fractions of oilseeds and of soya beans in particular. This may be desirable for a number of reasons:

1. A more concentrated protein source may be desirable for nutritional purposes.

2. The concentrated protein may possess enhanced functional properties in relation to its original form, offering applications in foodstuffs other than purely those of a protein source for nutritional purposes.

3. Concentration processes may in some cases simultaneously reduce the less desirable properties of the protein source (e.g. unpleasant flavour characteristics or anti-nutritional factors).

4. Concentration of the protein may result in added value products.

A major industry has developed around the processing of soya beans in order to concentrate their protein component (Smith & Circle 1977, Ohren 1981, Johnson & Kibuchi 1989). The most concentrated form of the protein, known as soya protein isolate, typically contains more than 90% of protein.

The best established method of soya protein isolate production is the so-called isoelectric precipitation process in which the soluble proteins of defatted soya beans are extracted into slightly alkaline water. The pH of the extract is then lowered to the

isoelectric point of the protein, at which it precipitates out, and is then separated from the remaining soluble non protein components. This process is used for the vast majority of commercially produced soya protein isolates and has been in use since the 1930's (Smith & Circle 1977).

An alternative separation process using ultrafiltration was first reported more than twenty years ago (Porter & Michaels 1970). A considerable amount of work in this field has been reported (Lawhon et al 1977, Lawhon et al 1979, Lawhon et al 1981) but it is not clear to what extent this technique is in commercial use.

A further potential process for soya protein extraction and concentration using so-called swollen gel technology was first reported some five years ago (Trank et al 1989). The process involves a novel and ingenious technology and was indicated to have a commercial potential.

Other methods of soya protein concentration such as grinding or air classification (Pfeiffer et al 1960) and ultrasonic extraction (Moulton & Wang 1982) have been reported but were found to be of only limited practical use.

It is clearly desirable for both scientific and commercial reasons to systematically compare the various extraction and concentration processes. While some data is available in literature for the comparison of experimentally produced ultrafiltration process isolates with commercially produced (presumably isoelectric process) isolates (Lawhon et al 1977, Lawhon et al 1979, Lawhon & Lusas 1984), no systematic study

has been published comparing properties of isolates produced from identical raw materials by the various processes available.

The objectives of this study were therefore:

1. To optimise the conditions under which the soluble proteins of defatted soya flake solids are separated from the insoluble portion, producing a solubilised soya extract which was then used as a standardised feed material for subsequent processing.
2. To optimise the separation and concentration conditions of the soluble protein fraction of the extract by means of:
    i. the isoelectric process
    ii. the ultrafiltration process
    iii. the swollen gel process.
3. To produce sufficient finished protein isolate by each of the processes for subsequent physical and chemical analysis.
4. To compare the functional properties of the products from each process.
5. To compare the levels of the various anti-nutritional factors present in soya in the products from each process.
6. To compare the composition of the proteins present in the products from each process.
7. To compare the economics of the three processes in terms of capital costs, process costs and finished product yields.

2. EXTRACTION OF SOLUBLE PROTEIN FRACTION FROM DEFATTED SOYA FLAKES.

2.1 LITERATURE REVIEW

A number of studies have been carried out on determining optimum solubilisation conditions for soya protein using defatted soya flakes as the starting material. The effect of pH and added salts on an otherwise standard extraction condition was studied (Smith & Circle 1938) and an alkaline pH was found to give best results. Lowest extraction rate was found at pH 4.2, however the addition of progressively increasing quantities of calcium chloride progressively nullified the effect of pH on protein solubility. Further studies on the effect of various salts on protein solubility (Smith et al 1938) indicated that the use of salts did not improve protein extraction levels over those obtained by water alone. It was also found that use of finely ground material slightly improved the extraction yield.

A detailed study of factors influencing solubility (Smith et al 1966) investigated the effect of pretreatment of the defatted meal, particle size of meal and extraction volumes at pH's of 7.2 and 6.5. It also investigated the extractability of protein from different soya bean strains. It was established that, providing well tempered and flaked soya beans were used as the source of defatted material, grinding of the meal gave no improvement in extraction yield and could even reduce the yield, due possibly to denaturation of the protein by heating during grinding. Two stage extractions using 20:1 and 10:1 water/flakes extraction ratios gave slightly improved yields over two stage extractions using 10:1 and 5:1 extraction ratios.

Extraction yields were slightly higher at pH 7.2 than at pH 6.5.

The combined effects of extraction ratio, method of agitation during solubilisation, extraction time and meal particle size on larger scale extraction efficiencies was studied by Cogan et al (1967). A total extraction ratio of 10:1 was found to be acceptable with higher ratios giving only a slight increase in yield. Vigorous agitation was also found to be desirable. Rapid solubilisation of protein occurred almost immediately and maximum solubilisation was reached after 30 minutes agitation. Particle size of the meal had little effect.

Other published studies using extraction of soluble protein to obtain a source of protein for further investigations (Lawhon & Lusas 1984, Nichols & Cheryan 1981, Okubo et al 1975) used arbitrarily chosen extraction conditions. All these extraction conditions fall broadly within the optimum parameters defined by previous studies and, in particular, indicate that two stage extraction procedures give improved results over those using a single extraction. Comparison of results from different studies is difficult as widely varying source materials were used.

2.2 EXPERIMENTAL PROCEDURE.

On the basis of previous studies in this field, it was decided to systematically study extraction conditions by choosing a set of standard conditions and varying individual parameters in turn while maintaining other parameters constant. Once the effect of the individual parameters had been determined, the composite effect of varying more than one parameter simultaneously could then be studied.

2.2.1 Standard Extraction Procedure

1. A quantity of 300g of defatted soya flakes was mixed with the required volume of water of the required temperature. The pH of the mixture was adjusted to the required value using 5N sodium hydroxide.
2. The vessel containing the mixture was placed in a waterbath set to the required temperature and stirred using a Heidolph laboratory stirrer at 1200 r.p.m. for the required time. The vessel was kept covered in foil to prevent moisture loss by splashing or evaporation.
3. The vessel was removed from the waterbath and the slurry was poured into a Martin Christ basket centrifuge fitted with a filtercloth bag. The slurry was centrifuged for 10 minutes at 3000 r.p.m.
4. The liquid extract was retained and the solid residue was redispersed in a further volume of water. The pH was readjusted to the required value using 5N sodium hydroxide.
5. The slurry was stirred for the required time as per (2) above
6. The slurry was re-centrifuged as per (3) above.
7. The second liquid extract was retained and pooled with the first extract.
8. The combined extracts were clarified using a De Laval 100 LPS laboratory separator.
9. Solids and protein contents for the combined clarified extracts were determined.

2.2.2 Determination of Extraction Yield

% Solids extracted

$$= \frac{\text{Total weight of 2 extracts x \% solids of combined extracts}}{\text{Weight of flakes used x \% solids of flakes.}} \times 100\%$$

% Protein extracted

$$= \frac{\text{Total weight of 2 extracts x \% protein of combined extracts}}{\text{Weight of flakes used x \% protein of flakes}} \times 100\%$$

Each 2 stage extraction was carried out in duplicate and average extraction figures for protein and solids were calculated.

2.3 DESCRIPTION OF VARIABLES.

A set of standard extraction conditions was chosen based on typical conditions used in earlier published studies, namely:

Volume of water used (1st extraction)	:	3000 ml.
Volume of water used (2nd extraction)	:	1500 ml.
pH of slurry (both extractions)	:	9.0
Extraction time (both extractions)	:	30 minutes
Extraction temperature (both extractions)	:	60°C

Particle size of flakes : 45-50% through T20 mesh (as received)

Conditions of extraction were varied as follows:

Volume of water used	(1st extraction)	3000 ml,	4500 ml,	6000 ml
pH of slurry	(both extractions)	9.0,	8.0,	7.0
Extraction time	(both extractions)	15 mins,	30 mins,	45 mins.
Extraction temperature	(both extractions)	50°C,	60°C,	70°C.

Particle size of flakes : as received (45 - 50% through T20 mesh)
ground to pass > 99% through T20 mesh

Duplicate two stage extractions were carried out, altering each variable in turn while maintaining all other variables as per the standard extraction procedure.

2.4 RAW MATERIAL USED.

Defatted soya flakes were obtained from National Protein, Potgietersrus. A single consignment of defatted soya flakes was used for the entire investigation to ensure consistency. Constant values for protein and moisture content were maintained by storing the flakes in tightly sealed woven polypropylene sacks with polythene liners at -15°C. Analysis of the defatted soya flakes gave the following results:

Solids content = 92.9%

Protein content = 46.2% (as is basis)

2.5 RESULTS

The effect of varying the extraction condition is shown below.

2.5.1 Effect of Varying Extraction Volume for 1st Extraction.

This is shown in Table 1.

Table 1. Effect of Extraction Volume on Extraction Yield.

Extraction volume (ml)	**3000 (standard)**	**4500**	**6000**
% Solids extracted	64.0	66.7	66.3
% Protein extracted	69.3	69.4	71.0

2.5.2 Effect of Varying pH of Slurry (both extractions)

This is shown in Table 2.

Table 2. Effect of pH on Extraction Yield.

Slurry pH	9.0 (standard)	8.0	7.0
% Solids extracted	64.0	63.2	61.3
% Protein extracted	69.3	68.2	66.3

2.5.3 Effect of Varying Extraction Time (both extractions)

This is shown in Table 3.

Table 3. Effect of Extraction Time on Extraction Yield.

Extraction time (mins)	15	30 (standard)	45
% Solids extracted	65.5	64.0	63.9
% Protein extracted	71.0	69.3	70.2

2.5.4 Effect of Varying Extraction Temperature (both extractions)

This is shown in Table 4.

Table 4. Effect of Extraction Temperature on Extraction Yield.

Extraction temperature (°C)	50	60 (standard)	70
% Solids extracted	65.1	64.0	61.1
% Protein extracted	70.1	69.3	64.9

2.5.5 Effect of Varying Particle Size of Flakes.

This is shown in Table 5.

Table 5. Effect of Particle Size on Extraction Yield.

Flake particle size	As received (standard)	>99% - T20 mesh
% Solids extracted	64.0	64.1
% Protein extracted	69.3	69.9

2.5.6 Comments on Extraction Results

The results obtained correlated well with those obtained in published studies and confirmed that only small changes in yield could be obtained by varying the different extraction parameters. Effects of the various parameters were as follows:

2.5.6.1. *Extraction volume* :

A slight increase in protein yields was noted with increased extraction volume. Solids yield increased slightly when the extraction volume increased to 4500ml but then decreased slightly at 6000ml extraction volume.

2.5.6.2. *pH:*

Best extraction results were obtained at pH 9. As expected, a reduction in yields was noted as the pH of the slurry was reduced. It might be expected that yield would increase further at higher pH's, but this was not pursued due to the potential risk of lysinoalanine formation.

2.5.6.3 *Extraction time:*

Increased extraction time reduced yields of both protein and solids. It appears that rapid solubilisation occurs.

2.5.6.4. *Extraction temperature:*

Yields were reduced with increasing temperature, however the improvement in yield observed at 50°C was not considered sufficient to compensate for the increased microbiological problems which could be expected at this temperature.

2.5.6.5. *Flake particle size:*

Only a very slight increase in yield was observed with the finer sized product.

2.6 EFFECT OF COMBINED VARIABLES

It was decided that only the combined effect of varying extraction time and first extraction volume required further investigation in view of the results obtained for pH, extraction temperature and particle size. A further series of extractions was therefore carried out as follows:

Standard conditions: Slurry pH : 9.0

Extraction temperature (both extractions): 60°C

Particle size of flakes: as received

Extraction volume (2nd extraction): 1500 ml.

The effect of the combined variables on extraction yields is shown in Table 6.

Table 6. Combined Effect of Extraction Volume and Extraction Time on Extraction Yield.

Extraction volume (1st extraction)	**Extraction time (both extractions)**	**% solids extracted.**	**% protein extracted**
4500 ml	15 mins.	66.6	71.2
4500 ml	45 mins.	67.0	72.2
6000 ml	15 mins.	67.5	70.1
6000 ml	45 mins.	68.3	72.3
c.f. standard extraction procedure.		64.0	69.3

Increases in yield were observed over the standard procedure. However, the use of larger extraction volumes and longer extraction times would significantly increase the capacity requirements for large scale processing. It is unlikely that these could be justified by the relatively small increases in yield that can be achieved.

2.7 EFFECT OF REVERSED EXTRACTION STAGES.

A final duplicate two stage extraction was carried out in which extraction volumes for the first and second extraction stages were reversed, namely with the following extraction conditions:

Volume of water used (1st extraction): 1500 ml

Volume of water used (2nd extraction): 3000 ml

pH of slurry (both extractions): 9.0

Extraction time (both extractions): 30 minutes

Extraction temperature (both extractions): 60°C

Particle size of flakes : as received

Results are shown in Table 7.

Table 7. Effect of Reversed Extraction Stages on Extraction Yield.

	Reversed extraction stages	c.f. Standard procedure
% Solids extracted	63.4	64.0
% Protein extracted	69.1	69.3

Theoretically, by using the reversed extraction volumes, a counter-current effect would be obtained by which a greater volume of water would be available to extract the more tightly bound soluble protein during the second stage of the extraction procedure. However, no improvement in yields over the standard procedure was observed.

2.8 CONCLUSION

It can be concluded that the only desirable variation from the original standard procedure would be a reduction in extraction times for both extractions from 30 minutes to 15 minutes on grounds of increased process efficiency rather than yield.

In view of the above, a set of revised standard extraction conditions was selected, taking into account process efficiency and yield considerations. These are listed in table 8 along with the original standard extraction conditions.

Table 8. Summary of Original and Revised Extraction Conditions.

	Original Standard	Revised Standard
Volume of water used (1st extraction)(ml)	3000	3000
Volume of water used (2nd extraction)(ml)	1500	1500
pH of slurry (both extractions)	9.0	9.0
Extraction time (both extractions)(mins)	30	15
Extraction temperature (both extractions) (°C)	60	60
Particle size of flakes	As received	As received

A trial bulk extraction using the above conditions was carried out, using 5.5 kg. of defatted soya flakes, 55 litres of water for the first extraction and 27.5 litres of water for the second extraction. Extractions were carried out in an 80 litre stainless steel vessel using a Lightning mixer. Separation was carried out using continuous centrifugation in a Martin Christ centrifuge and the final clarification of the extract was carried out using a Westphalia SA 1-01-175 separator. Yield figures are shown in Table 9.

Table 9. Comparison of Yields from Standard Extraction Procedure and Bulk Extraction.

	Bulk extraction	c.f. Standard procedure
% Solids extracted	58.0	64.0
% Protein extracted	62.2	69.3

The loss in extraction efficiency relative to the standard extraction procedure can be attributed to the practical difficulties associated with handling larger quantities of material and, in particular, the centrifugation of larger quantities of slurry. Due to the wide variety of conditions used in existing published studies, comparison of results with those from existing published studies is difficult. However Lawhon & Lusas (1984) reported a 55.0% yield of extracted solids for a single stage extraction using a 1:15 solids/water extraction ratio, a pH of 8.0, a temperature of 65°C and an extraction time of 40 minutes.

3. ISOELECTRIC PRECIPITATION OF SOYA PROTEIN.

3.1 LITERATURE REVIEW

The solubility characteristics of soya protein at varying pH values are well known (Smith & Circle 1938). A subsequent study (Smith & Circle 1939) systematically studied the effect of different pH's and pH adjusting agents on the proportions of protein precipitated out. The results obtained in both studies indicated an isoelectric point of around pH 4.2. Both original extraction conditions for the protein and type of acid used had little effect on the degree of solubilisation.

A more recent study on extraction conditions (Cogan et al 1967) confirmed that type of acid used for isoelectric precipitation had little effect. The same study also found that additions of calcium chloride to boiling extracts gave yields of precipitated protein comparable to those obtained by isoelectric precipitation.

Detailed studies have been carried out on solubility characteristics of different soya bean protein fractions produced isoelectrically (Lilford & Wright 1981, van Megen 1974) but these are of only limited value for industrial purposes, where maximisation of yield is of primary importance.

3.2 EXPERIMENTAL

It was decided to carry out a simple study to confirm previous results namely to optimise pH conditions for maximum isoelectric precipitation and to evaluate hydrochloric, sulphuric and phosphoric acids as pH adjusters.

3.2.1 Experimental Procedure.

1. A standard soya protein extract was prepared by the revised standard procedure discussed earlier. Solids and protein contents of the extract were determined.

2. 100 ml aliquots of extract were adjusted to varying pH's using 2N hydrochloric acid, 2N sulphuric acid and 1M phosphoric acid respectively. In each case the samples were stirred vigorously during addition of the acid and the sample was allowed to stand for 2 minutes after which the pH was readjusted to the required value if necessary. The volume of acid required for pH adjustment was noted.

3. The samples were then poured into 250 ml centrifuge tubes and centrifuged for 10 minutes at 2000 rpm.

4. A portion of the supernatant from each sample was drawn off, filtered through a 42 Whatman filter paper and analyzed for protein content.

5. The percentage of the total protein in solution at each pH was determined in relation to the protein content of the original extract and adjusted to compensate for the varying sample volumes caused by the varying amounts of acid added.

6. Two determinations were carried out at each pH for each acid and the average percentage of solubilised protein reported.

3.3 RESULTS

Results for the three acids are summarised in Tables 10, 11 and 12.

Table 10. Percentage Protein Solubilised at Varying pH's Using 2N Hydrochloric Acid.

pH	5.5	5.0	4.75	4.5	4.25	4.0	3.5	3.0
% Protein in solution	99.0*	78.9*	17.8	12.1	11.7	12.1	12.1	21.1

Table 11. Percentage Protein Solubilised at Varying pH's Using 2N Sulphuric Acid.

pH	5.5	5.0	4.75	4.5	4.25	4.0	3.5	3.0
% Protein in solution	99.2*	33.2*	17.4	13.4	12.6	11.7	12.1	14.6

Table 12. Percentage Protein Solubilised at Varying pH's Using 1M Phosphoric Acid.

pH	5.5	5.0	4.75	4.5	4.25	4.0	3.5	3.0
% Protein in solution	99.6*	34.6*	17.1	13.8	11.4	11.4	11.0	16.7

*At pH's 5.5 and 5.0 the insoluble material was so fine that a clear supernatant could not be achieved, even after filtration. Results obtained at these pH's are therefore estimates, however this occurred at pH's well above the isoelectric point and results obtained over the critical pH range of 4.5 - 3.5 were not affected in this way.

3.4 CONCLUSIONS

While the results obtained are broadly in agreement with the results of Smith & Circle (1939) and Cogan et al (1967), slight differences in degree of solubility between samples using the different acids are observed. In particular, Table 3 shows that phosphoric acid at pH's 4.25, 4.0 and 3.5 gives lower proportions of protein in solution than those obtained with hydrochloric and sulphuric acids. The low protein solubilities for all three acids also persist over a slightly wider range of pH than that observed in other studies.

However, the differences observed are so small that it can be concluded that, providing a pH in the range 4.25 - 3.5 is achieved, choice of acid for precipitation is governed by cost of the acid itself rather than any other parameters. Current costs for industrial quantities of the three acids are:

Sulphuric acid: R1800/ton.

Hydrochloric acid: R 955/ton.

Phosphoric acid: R3200/ton.

Hydrochloric acid was therefore adopted for subsequent work on the isoelectric process.

4. PREPARATION OF SOYA PROTEIN ISOLATE BY ISOELECTRIC PRECIPITATION.

Following the investigations into optimum conditions for protein solubilisation and isoelectric precipitation, samples of soya protein isolate were prepared on a pilot plant scale. The procedure used was as follows:

4.1 PROTEIN SOLUBILISATION.

Although a two stage extraction process gives a better yield than a single stage process, it is unwieldy and impractical for pilot scale processing. A single stage extraction was therefore used with the following conditions:

Weight of flakes: 4.2 kg
Volume of water: 56ℓ
) This extraction ratio was chosen as a compromise between the original single and double extraction volumes.

pH adjusted to 9.0 with 5N sodium hydroxide

Temperature: 55° C - this was adopted as large volumes of tap water of this temperature were readily available.

Extraction time: 15 minutes

4.2 REMOVAL OF INSOLUBLE PORTION

Due to the large volumes of material involved and limited centrifugation capacity, centrifugation of the slurry was excessively time consuming and an attempt at separation using a hydrocyclone was also found to be impractical. It was then established that straining of the slurry through a 150 micron screen gave a reasonably quick separation of the soluble and insoluble fractions and this method was adopted for subsequent work.

4.3 ISOELECTRIC PRECIPITATION.

The pH of the soluble fraction was adjusted to 4.2 with concentrated hydrochloric acid. The resulting slurry was allowed to stand overnight and the supernatant liquid was then decanted off.

4.4 WASHING OF PRECIPITATED MATERIAL

A volume of water equivalent to approximately 1.5 x the volume of supernatant decanted at the previous stage was then added to the protein sludge. The slurry was stirred vigorously to disperse the remaining insoluble components.

4.5 SEPARATION OF PRECIPITATED MATERIAL.

Initially separation was carried out using a Westphalia S.A. 1-01-175 separator in clarifier mode. However considerable difficulties were experienced due to the erratic mechanical performance of the separator and the loss of product through clogging of the separation bowl. Use of the Westphalia unit was therefore abandoned and separation was carried out by:

i. Allowing the washed slurry to stand and settle out.

ii. Decanting off the supernant.

iii. Straining the resulting sludge through a 150 micron screen - this removed most of the solid material.

iv. Straining the liquid fraction from stage iii through a 74 micron screen to remove fine material.

4.6 RE-SOLUBILISATION OF PROTEIN

The pH of the resulting sludge was adjusted to 8.0 using 5N sodium hydroxide. The

resulting mixture was stirred vigorously until full re-solubilisation had occurred.

4.7 SPRAYDRYING OF PROTEIN ISOLATE FROM ISOELECTRIC PROCESS.

The protein solution was heated to 55°C and filtered through 833 micron mesh to remove residual insoluble material. This solution was spraydried on a Nebulosa pilot plant spry dryer fitted with a nozzle atomiser. Conditions were as follows:

Atomising pressure:	200 KPa
Inlet air Temperature:	143 - 160°C
Outlet air temperature:	70 - 85°C
Throughput:	30 - 50 ml solution per minute.
Estimated evaporation capacity:	1.6 - 2.7 kg/hr.

Considerable difficulty was experienced at the spraydrying stage due to the very high viscosity of the protein solution which necessitated spraydrying at a feed solids content of 8.5 - 9.0%. Even at this solids level, blockages of the feed nozzle and buildup of solid material in the drying chamber and connecting pipework caused considerable problems and it was impossible to spraydry for more than 4 hours at a time, after which cleaning of the dryer became necessary. This resulted in very low yields of dried product which cannot be considered in any way representative of true manufacturing conditions.

Three complete runs based on the above process were carried out.

4.8 RESULTS

4.8.1 Analysis of Finished Product.

Chemical analyses of the isoelectric process slurries prior to re-solubilisation and the finished spraydried products are shown in Table 13 and Table 14.

Table 13. Analysis of Isoelectric Process Slurries.

	Batch 1	Batch 2	Batch 3
% Protein	7.30	10.29	9.75
% Solids	8.67	11.48	11.15
% Protein (dry basis)	84.2	89.6	87.4

Table 14. Analysis of Sprydried Product ex Isoelectric Process

	Batch 1	Batch 2	Batch 3
% Protein	76.60	77.86	77.69
% Solids	90.86	89.56	92.97
%Protein (dry basis)	84.3	86.9	83.6

4.8.2 Calculation of Yields

As explained above, the unrepresentative nature of the spraydrying process necessitated the calculation of yields in a number of different ways:

$$\text{Dry solids yield (Isoelectric process only)} = \frac{\text{Wt of slurry before resolubilising x \% solids of slurry}}{\text{Wt of extract used x \% solids of extract}} \times 100\%$$

$$\text{Protein yield (Isoelectric process only)} = \frac{\text{Wt of slurry before resolubilising x \% protein of slurry}}{\text{Wt of extract used x \% protein of extract}} \times 100\%$$

$$\text{Dry solids yield (extraction/ isoelectric)} = \frac{\text{Wt of slurry before resolubilising x \% solids of slurry}}{\text{Wt of flakes in original extraction x \% solids of flakes}} \times 100\%$$

$$\text{Protein yield (extraction/ isoelectric)} = \frac{\text{Wt of slurry before resolubilising x \% protein of slurry}}{\text{Wt of flakes in original extraction x \% protein of flakes}} \times 100\%$$

$$\text{Dry solids yield (spraydrying)} = \frac{\text{Wt of dried product x \% solids of dried product}}{\text{Wt of spraydryer feed x \% solids of feed}} \times 100\%$$

$$\text{Protein yield (spraydrying)} = \frac{\text{Wt of dried product x \% protein of dried product}}{\text{Wt of spraydryer feed x \% protein of feed}} \times 100\%$$

4.8.3 Yields

The percentage yields obtained using the above methods are summarised in Table 15.

Table 15. Yields for Isoelectric Process Samples.

		Batch 1	Batch 2	Batch 3
Isoelectric process only	**Dry solids yield** **Protein yield**	50.4 78.0	54.3 83.4	54.1 83.6
Extraction/ Isoelectric process	**Dry solids yield** **Protein yield**	24.9 42.1	27.4 49.3	27.4 48.2
Spray-drying	**Dry solids yield** **Protein yield**	36.7 36.7	40.4 39.2	58.7 56.1

4.9 DISCUSSION

In spite of the fairly crude methods used for separation, yields for the extraction / isoelectric process were fairly consistent with batches 2 and 3 in particular giving good replication. It is likely that yields for this stage could be improved by:

i. adopting a two stage extraction process

ii. use of more sophisticated centrifugal separation processes to increase the yield of liquid obtained from the extraction process.

iii. use of more sophisticated centrifugal separation processes to minimise loss of the insoluble protein fraction.

Yields for the spraydrying stage show greater variation, indicating the practical difficulties of spraydrying the protein solution using the available equipment.

It should also be noted that, for the purposes of this study, comparison of yields from the extraction/isoelectric process is of greater relevance than those from the spraydrying stage, as the spraydrying stage is common to all the three protein separation methods used.

5. PRODUCTION OF SOYA PROTEIN ISOLATE USING ULTRAFILTRATION

5.1 LITERATURE REVIEW

The potential of membrane processing for extraction of protein from soyabeans was first reported over twenty years ago (Porter & Michaels 1970), when it became apparent that the sizeable differential in molecular weights between the protein fractions (molecular weights mainly greater than 20000) and non-protein fractions (molecular weights less than 1000) of the soluble components of soya beans would enable the two fractions to be separated by means of a semi-permeable membrane. Investigations as to the commercial viability of the process soon followed (Frazeur & Huston 1973, Goodknight et al 1976),

Very extensive work in this field has been carried out at the Texas A & M University Food Protein Research and Development Centre and at the University of Illinois. Areas studied have included comparison of different membrane configurations (Lawhon et al 1978), ultrafiltration systems from different manufacturers (Lawhon et al 1977) and the effects of parameters such as pH (Omosaiye et al 1978), temperature (Hensley et al 1977), feed concentration (Lawhon et al 1978) and pressure (Hensley et al 1977) on the performance of the systems. Most of the experimental work has been carried out using a batch diafiltration process in which the volume of the solution being processed is maintained constant by addition of water to compensate for the loss of the liquid containing the low molecular weight solutes, thus causing a progressive increase in the protein content on a dry solids basis in the remaining solution. The benefits of this approach have been discussed (Lawhon & Lusas 1984). Very extensive volumes of data are available and it is concluded (Lawhon et al 1979,

Lawhon & Lusas 1984) that the use of ultrafiltration systems provides a technically feasible alternative to the established isoelectric precipitation method of soya isolate production. An economic evaluation of the ultrafiltration process relative to the isoelectric process (Hensley & Lawhon 1979) indicates that the ultrafiltration process is commercially viable. It is believed that certain manufacturers have subsequently implemented the ultrafiltration process for soya isolate but, due to considerations of commercial confidentiality, further details are not available.

5.2 EXPERIMENTAL

Experimental work was carried out using an Osmonics Osmo-17T-UF-PES pilot scale ultrafiltration unit with a spirally wound membrane configuration. To enable a direct comparison to be made with the isoelectric process, the feed solution used was identical to that used for isoelectric processing, using defatted soya flakes from the same consignment and an identical extraction procedure.

Two batches of product were extracted for each ultrafiltration run, giving typical ultrafiltration batch sizes of 90 - 95 litres. A schematic diagram of the apparatus used is shown in Fig.1.

Due to the size of the apparatus, all experimental work was carried out using a combined ultrafiltration/diafiltration procedure as single stage ultrafiltration would not have achieved the required separation.

The object of the experimental work was to obtain a set of optimum conditions for production of a soya isolate solution for subsequent spraydrying. These were to be assessed in terms of:

i. efficiency of separation of protein and non protein components (measured in terms of protein on a dry solids basis in the finished product)

ii. speed of operation (the shortest possible processing time to produce a desirable product is clearly desirable).

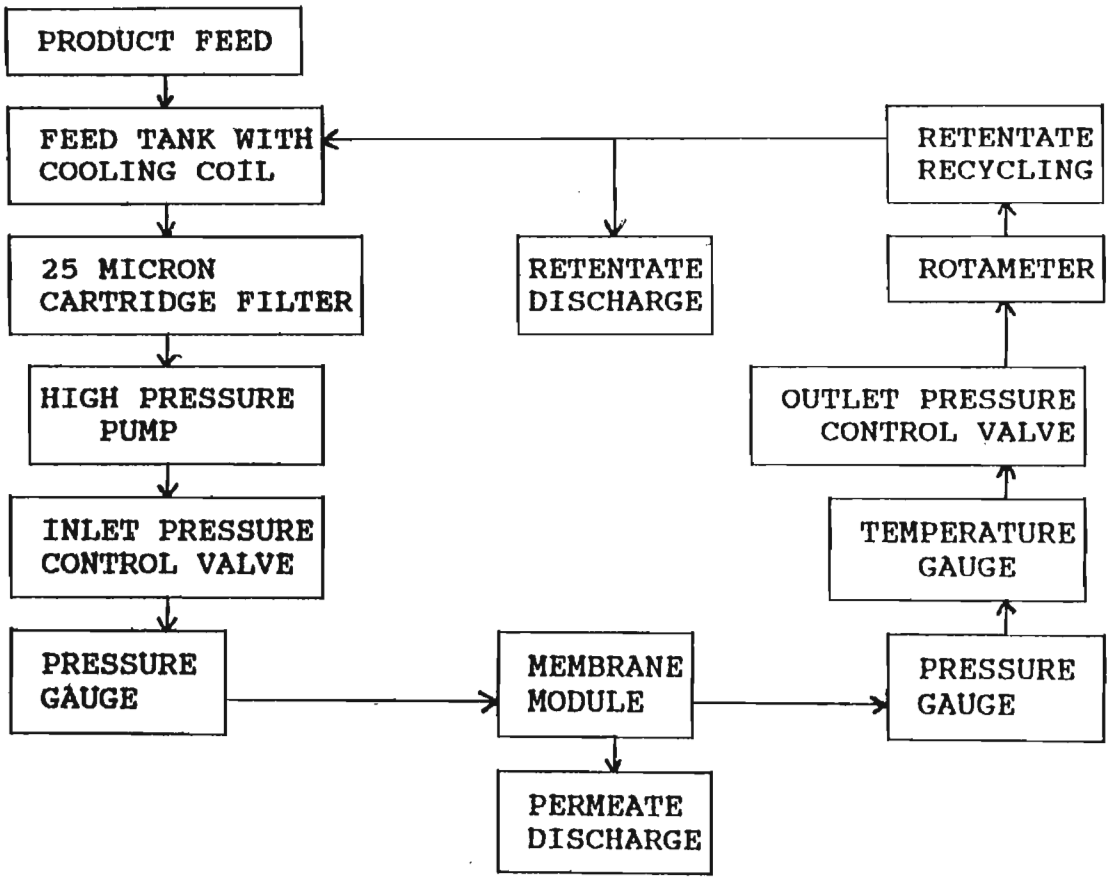

Fig. 1. Ultrafiltration Unit.

5.3 CHOICE OF PARAMETERS TO BE STUDIED.

Certain parameters remained unchanged throughout the test runs:

i. Initial solution pH was maintained at 8.5 - 9.0 as lower values would reduce protein solubility while higher values could cause formation of lysinoalanine

ii. Temperature was maintained between 55°C and 60°C as lower temperatures were likely to result in microbiological spoilage problems whereas higher temperatures could cause protein denaturation and lysinoalanine formation.

The following parameters were selected for further study:

i. Choice of membrane pore size. Membranes with molecular weight cutoffs (MWCO'S) of 80000 - 120000 and 40000 - 60000 respectively were evaluated.

ii. Pressure drop across the membrane module.

ii. Various combinations of preconcentration/diafiltration/final concentration times.

5.4 TEST PROCEDURE

The procedure used involves the repeated cycling of the test solution through the apparatus and collection of retentate samples for analysis at regular intervals. Pressure drop and flow rates were controlled by use of the two regulatory valves. Temperature of the system was maintained at 55 - 60°C by periodic cooling of the feed tank by means of a cooling coil. Solution volume was maintained constant during diafiltration by addition of water to the feed tank. Product yield when required was obtained by determining the weight of solution remaining in the feed tank to which the weight of solution (approximately 7kg) retained in the apparatus was added.

5.4.1. Tests to Determine Choice of Membrane.

Two test runs were carried out using spiral-wound membranes of 80000 - 120000 and 40000 - 60000 MWCO'S. Membranes were supplied by Messrs. Osmonics Inc. The tests comprised 5 hours diafiltration with a pressure drop of 1.3 bar followed by concentration to a point where the temperature could no longer be kept below 60°C. Concentration times were 2 hours for the 80000 - 120000 MWCO membrane test and 1 hour 20 minutes for the 40000 - 60000 MWCO membrane test.

Results, expressed in terms of analysis figures for retentate samples taken at half hourly intervals, are shown in Table 16.

Table 16. Effect of Membrane MWCO on Changes in Retentate Composition.

Time (mins)	80000-120000 MWCO Membrane			40000 - 60000 MWCO Membrane		
	% Protein	% Solids	% Protein Dry Basis	% Protein	% Solids	% Protein Dry Basis
Initial	2.72	4.60	59.1	2.33	4.21	55.3
30	2.52	3.95	63.8	2.34	3.60	65.0
60	2.51	3.55	70.7	2.33	3.26	71.5
90	2.44	3.43	71.1	2.28	3.06	74.5
120	2.30	2.95	78.0	2.37	3.02	78.5
150	2.40	3.07	78.0	2.37	2.92	81.2
180	2.47	3.01	82.1	2.66	3.26	81.6
210	2.47	2.98	82.9	2.65	3.17	83.6
240	2.36	2.88	81.9	2.69	3.20	84.1
270	2.32	2.91	79.7	2.60	3.09	84.1
300	2.43	2.79	87.1	2.72	3.18	85.5
300	END DIAFILTRATION - BEGIN CONCENTRATION					
330	2.95	3.41	86.5	4.50	5.24	85.9
360	3.62	4.29	84.4	7.89	8.93	88.4
390	5.10	5.82	87.6	11.11	12.50	88.9
420	7.51	8.44	89.0			

The results are shown graphically in Fig.2. It can be seen that, while performance during the diafiltration stage is broadly similar for both membranes, the 40000 - 60000 MWCO membrane gives a much better performance during the concentration stage in terms of speed of concentration and final solids achieved (which clearly must be as high as possible in order to minimise the drying load during the subsequent spraydrying). This can be explained in terms of the molecular weights of the material present in solution after diafiltration - very little protein of MW 40000 - 60000 is likely to be present whereas an appreciable amount of protein with MW of 80000 - 120000 will be present and this will tend to clog the pores of the higher MWCO membrane but simply pass over the pores of the lower MWCO membrane.

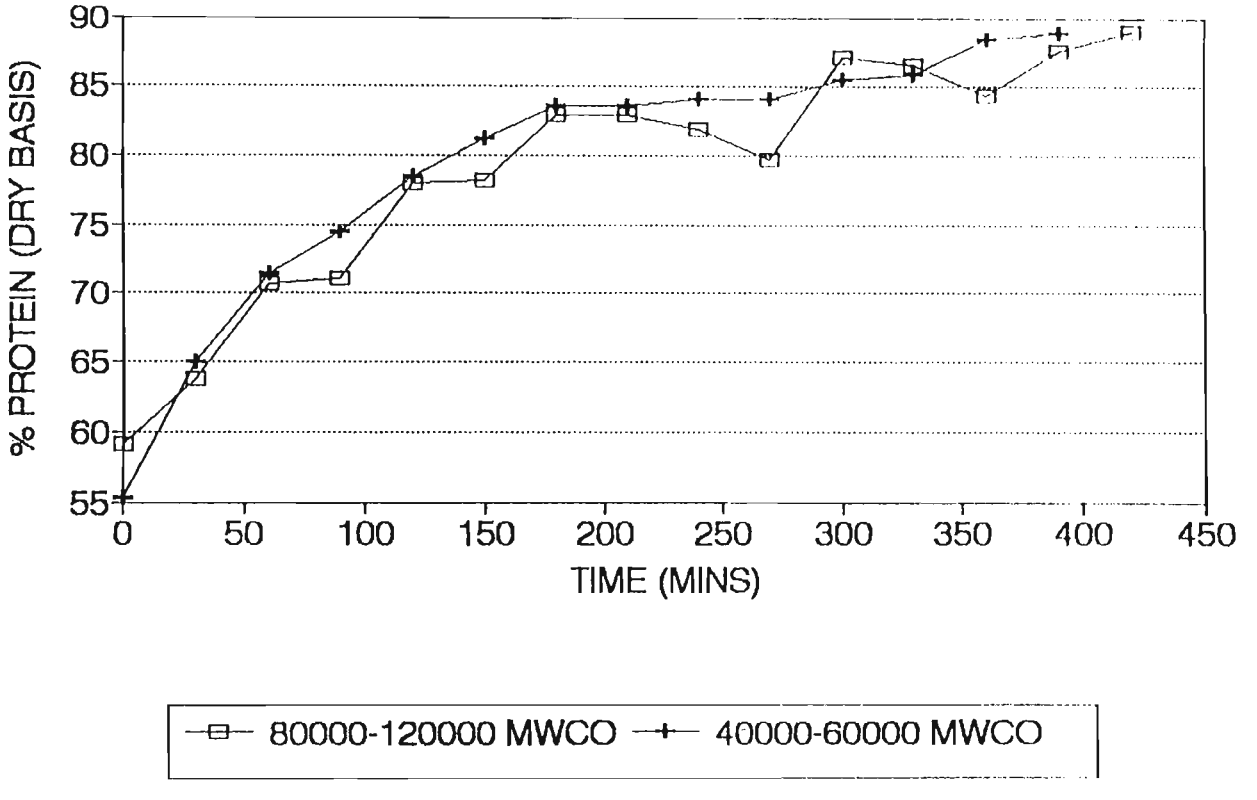

Fig. 2. Ultrafiltration. Effect of Membrane MWCO.

It was consequently decided to use the 40000 - 60000 MWCO membrane for all subsequent test work. It can also be seen from the graph that protein percentage on a dry basis showed virtually no increase between 180 minutes and 300 minutes, indicating very limited separation of protein from non-protein components during the latter stages of diafiltration. For subsequent tests, the diafiltration time was therefore reduced from 300 minutes to 180 minutes.

5.4.2 Test to Determine Optimum Pressure Drop.

In theory, increased pressure drop across the membrane module should increase separation efficiency but in practice this advantage needs to be offset against the increased wear on the membrane resulting from higher pressures. Three test runs were undertaken using the 40000 - 60000 MWCO membrane with pressure drops of 1.8, 1.3 and 1.0 bar respectively. In each case a diafiltration stage of 3 hours was followed by a concentration stage.

Results, expressed in terms of analysis figures for retentate samples taken at half hourly intervals, are shown in Table 17.

Table 17. Effect of Pressure Drop on Changes in Retentate Composition.

(% protein, % solids, % protein dry basis.)

	1.8 BAR			1.3 BAR			1.0 BAR		
Time (mins)	% Prot	% Slds	% Prot dry basis	% Prot	% Slds	% Prot dry basis	% Prot	% Slds	% Prot dry basis
Initial	2.71	4.43	61.1	2.52	4.40	57.3	2.67	4.54	58.8
60	2.69	3.51	76.6	2.54	3.52	72.2	2.63	3.63	72.5
120	2.53	3.00	84.3	2.47	3.13	78.9	2.55	3.19	79.9
180	2.47	2.83	87.3	2.42	2.91	83.2	2.52	3.04	82.9
180	END DIAFILTRATION - BEGIN CONCENTRATION								
260	13.67	15.42	88.7						
280				11.19	12.57	89.0			
285							12.70	14.24	89.2

The results are shown graphically in fig.3. As expected the higher pressure drop gave improved separation efficiency during diafiltration however, by using a slightly longer concentration time at lower pressure, an equally acceptable final separation was achieved. In view of the membrane wear consideration mentioned above, it was decided to use a 1.0 bar pressure drop during subsequent tests.

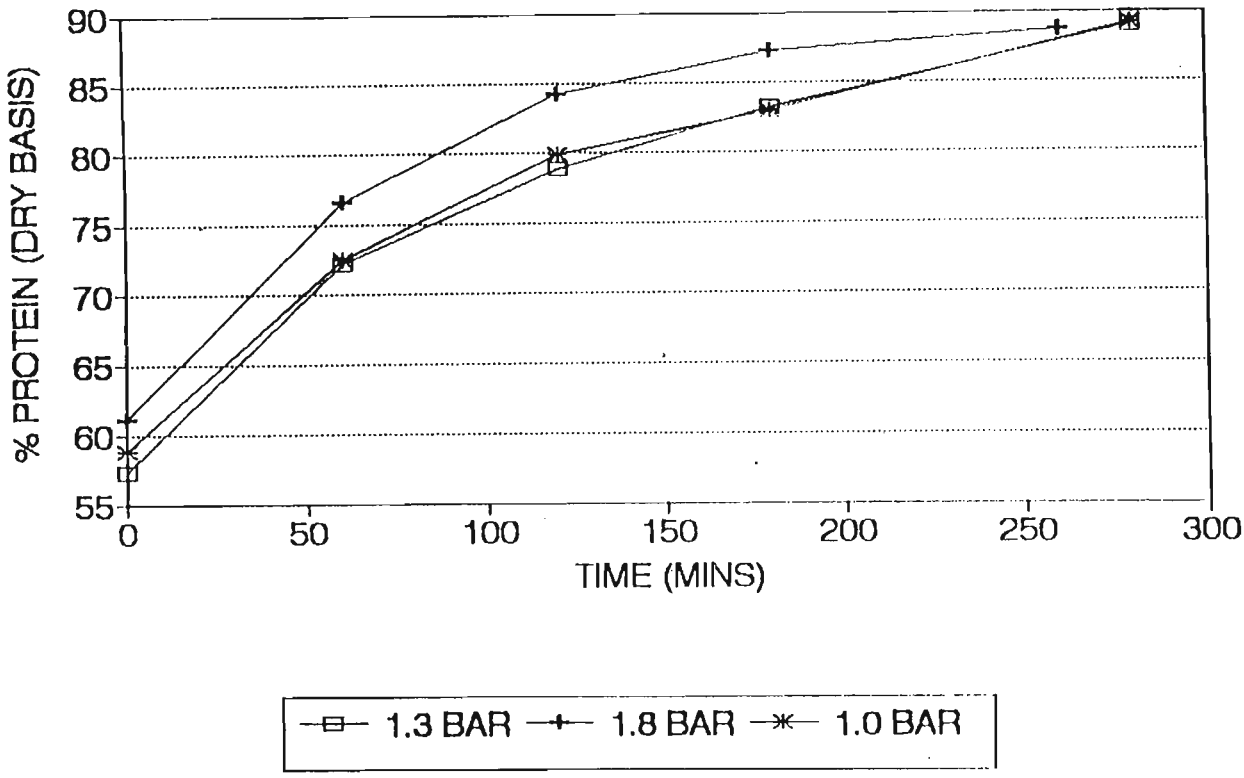

Fig. 3. Ultrafiltration. Effect of Pressure Drop.

5.4.3 Test to Determine Optimum Concentration/Diafiltration Sequence.

Up to this stage diafiltration had been carried out by maintaining the initial volume of material throughout the diafiltration stage. It was decided to carry out a preconcentration stage prior to diafiltration so that the efficiency of the diafiltration stage could be enhanced due to the lower volume of material processed and consequent greater number of process cycles for the same time.

A test was therefore carried out using a 40000 - 60000 MWCO membrane with 1.0 bar pressure drop in which the initial volume of liquid was reduced by half by a preconcentration step. This took 50 minutes and was followed by standard diafiltration for a further 2 hours 10 minutes. A further final concentration stage then followed.

Results for this test, compared to those for the previous test at 1.0 bar pressure drop, expressed in terms of analysis figures for retentate samples taken at regular intervals are shown in Table 18.

Table 18. Effect of Preconcentration on Changes in Retentate Composition.

	DIAFILTRATION/ CONCENTRATION			PRECONCENTRATION/ DIAFILTRATION/ CONCENTRATION		
Time (mins)	**% Protein**	**% Solids**	**% Protein dry basis**	**% Protein**	**% Solids**	**% Protein dry basis**
Initial	2.67	4.54	58.8	2.54	4.34	58.5
50	-	-	-	5.23	7.30	71.6
50				**End Preconcentration - Begin Diafiltration**		
60	2.63	3.63	72.5	-	-	-
120	2.55	3.19	79.9	5.01	5.87	85.3
180	2.52	3.04	82.9	5.39	6.11	88.2
180	**End Diafiltration - Begin Concentration**					
235	-	-	-	11.66	12.96	90.0
285	12.70	14.24	89.2	-	-	-

Results are summarised in Fig.4.

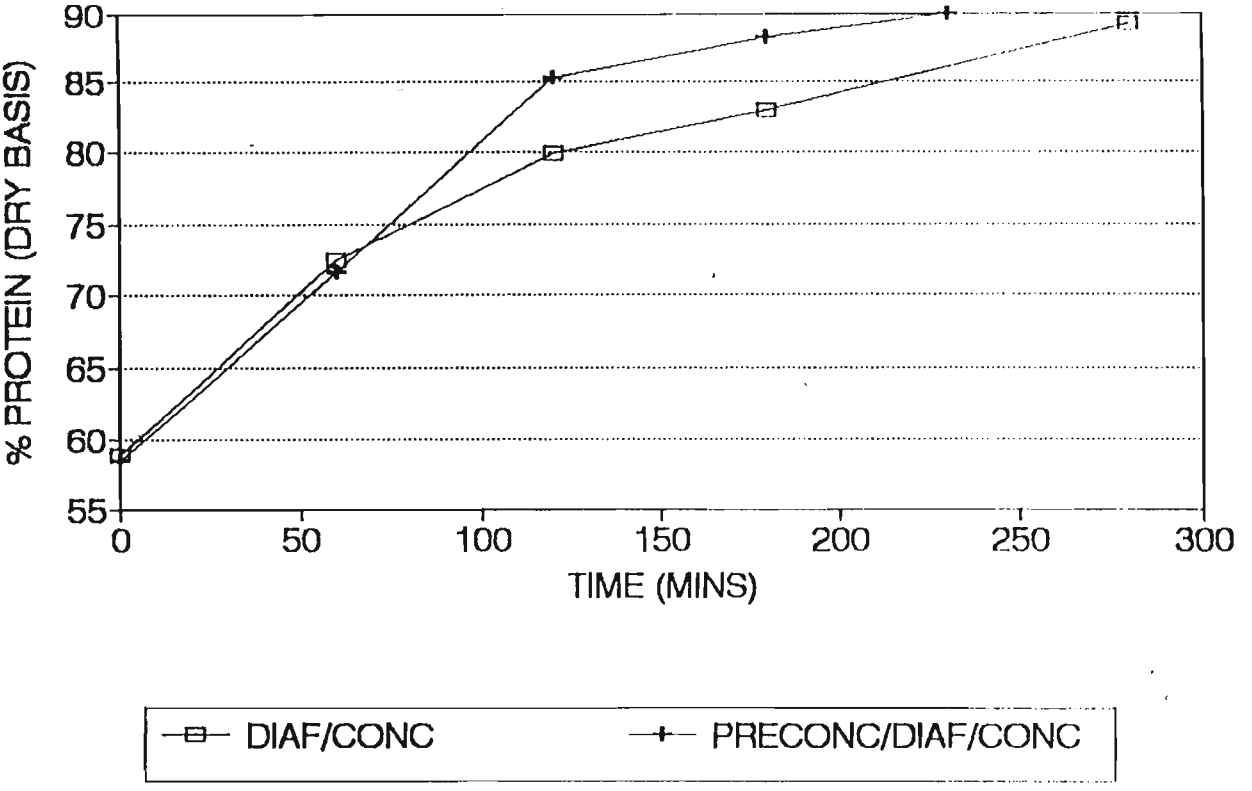

Fig. 4. Ultrafiltration. Effect of Pre-Concentration.

The improved and faster separation produced by diafiltration at higher concentrations is immediately apparent.

5.4.4 Test to Establish Effect of Concentration/Dilution vs. Diafiltration and Effect of Increased Pressure Drop During Final Concentration.

A subsequent test was undertaken to establish the effect of several bulk re-dilutions during the processing of pre-concentrated material in order to compare this to diafiltration at constant volume. It was also suggested that an increase in pressure drop during the final concentration stage only would increase process efficiency and,

as the higher pressure would only be used for a limited time, membrane wear would be minimised.

The following test sequence was therefore used:

Concentrate to half original volume then redilute to original volume (0 - 50 mins)

Re-concentrate to half volume then redilute to original volume . . (50 - 100 mins)

Re-concentrate to half volume . . . . . (100-150 mins)

Increase pressure drop to 1.8 bar and concentrate further . . . . . (150-200 mins)

Results for this test (designated "concentration/bulk dilution/concentration" test) relative to those for the previous preconcentration/diafiltration/concentration test, expressed in terms of analysis figures for retentate samples taken at regular intervals, are shown in Table 19.

Table 19. Effect of Bulk Dilution on Changes in Retentate Composition.

	PRECONCENTRATION/ DIAFILTRATION/ CONCENTRTION			CONCENTRATION/ BULK DILUTION/ CONCENTRATION		
Time (mins)	% Protein	% Solids	% Protein Dry Basis	% Protein	% Solids	% Protein Dry Basis
Initial	2.54	4.34	58.5	2.57	4.33	59.4
50	5.23	7.30	71.6	2.58	3.62	71.3
100	-	-	-	2.54	3.24	78.4
120	5.01	5.87	85.3	-	-	-
150	-	-	-	5.04	5.87	85.9
150				Begin Final Concentration		
180	5.39	6.11	88.2			
180	Begin Final Concentration					
200				12.93	14.58	88.7
235	11.66	12.96	90.0			

Results are summarised graphically in Fig. 5.

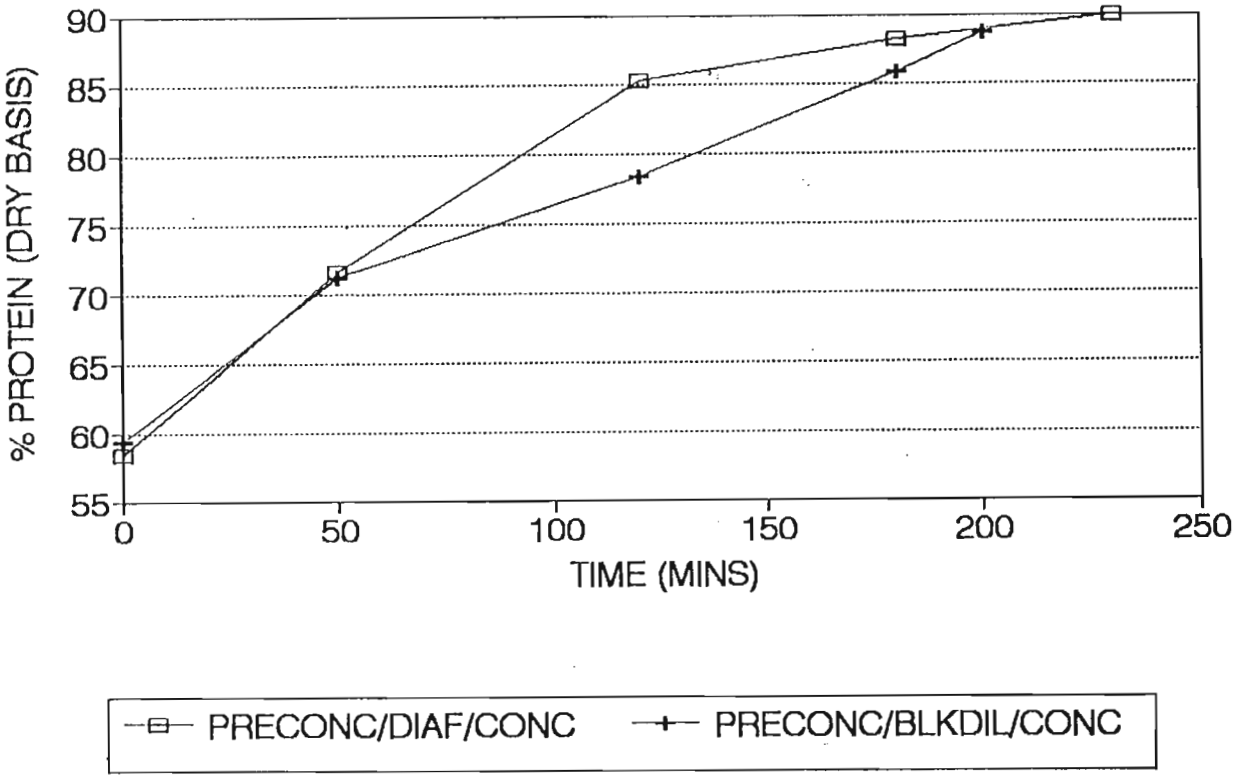

Fig. 5. Ultrafiltration. Effect of Bulk Dilution.

It can be seen that efficiency of separation is not as good for the concentration/ dilution/concentration procedure as for the preconcentration/diafiltration/concentration procedure. However, use of the higher pressure drop during the final stage of the concentration/dilution/concentration procedure improves the rate of increase of percentage solids (i.e. final concentration is more rapid using the higher pressure drop).

5.5 CHOICE OF STANDARD PROCESS CONDITIONS.

The following set of standard process conditions were therefore chosen:

Batch size	90 kg.
Temperature	55° - 60°C
Initial pH	8.5 - 9.0
Pressure drop (preconcentration/diafiltration stages)	1.0 bar
Pressure drop (final concentration stage)	1.8 bar
Pre-concentrate to 50% of original volume	(50 mins)
Diafiltration	(2 hours 10 mins)
Final concentration until temperature rises excessively.	

5.6 PROCESSING USING OPTIMISED CONDITIONS.

Three test runs were conducted using the standard process conditions. Results, expressed in terms of analysis figures for retentate samples taken at regular intervals are shown in Table 20.

Table 20. Analysis Figures for Samples Produced in Repeat Runs Under Optimised Conditions.

(% protein, % solids, % protein dry basis.)

	BATCH 1			BATCH 2			BATCH 3		
Time (mins)	% Prot.	% Slds	% Prot. dry basis	% Prot.	% Slds	% Prot. dry basis	% Prot.	% Slds	% Prot. dry basis
Initial	2.62	4.29	61.1	2.59	4.30	60.2	2.55	4.26	59.9
50	5.10	7.05	72.3	5.22	7.15	73.0	5.27	7.19	73.3
50	END PRECONCENTRATION - BEGIN DIAFILTRATION								
120	4.51	5.45	82.8	4.80	5.68	84.5	4.66	5.49	84.9
180	4.37	5.06	86.4	5.10	5.68	89.8	5.20	5.85	88.9
180	END DIAFILTRATION - BEGIN FINAL CONCENTRATION								
225	12.84	13.99	91.8	13.20	14.40	91.7	12.83	14.05	91.3

Results, summarised in Fig.6, are highly consistent and show the effect of the higher pressure drop during the final concentration stage which further improves separation efficiency relative to previous runs.

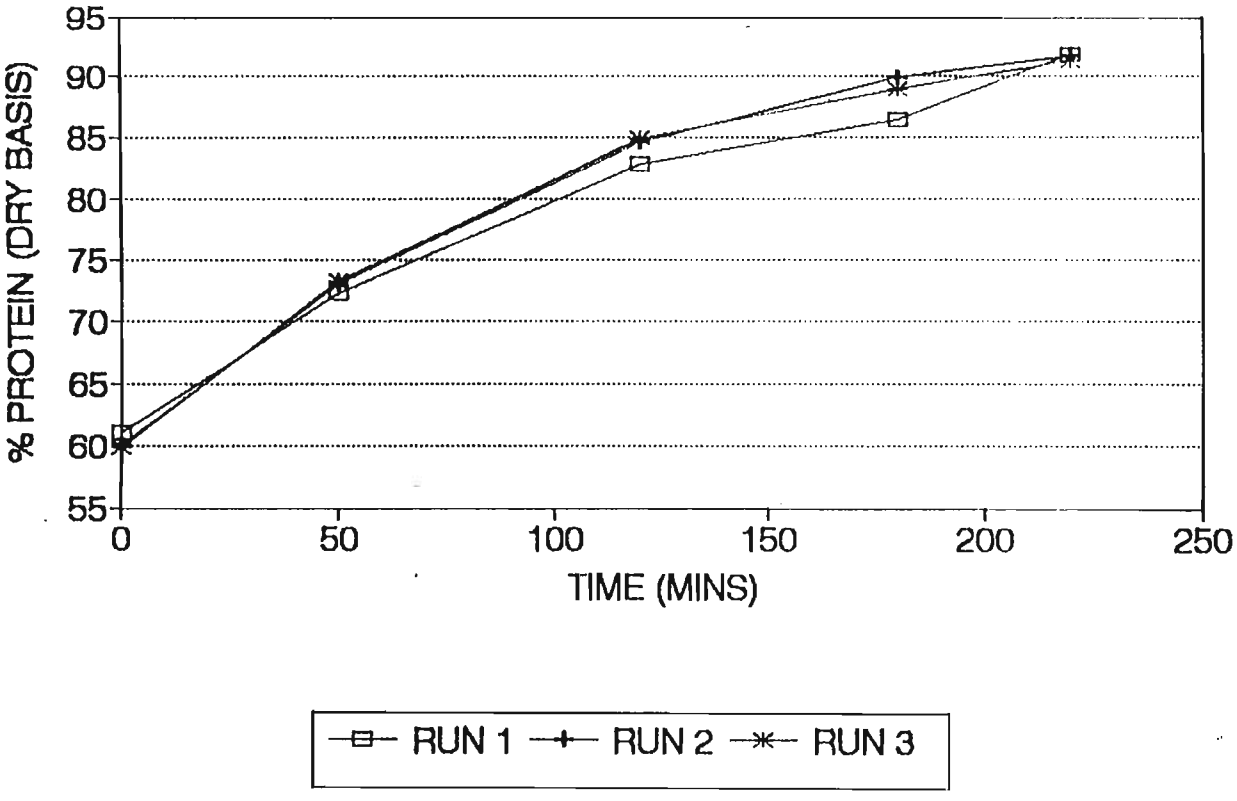

Fig. 6. **Ultrafiltration.** Processing Using Optimised Conditions.

5.7 SPRAYDRYING OF PROTEIN ISOLATE FROM ULTRAFILTRATION PROCESS.

The protein solutions were pre-heated to 55°C and mixed using a Silverson mixer to ensure a uniform mixture. They were then spraydried in a Nebulosa pilot plant spray dryer fitted with a nozzle atomiser. Conditions were as follows

Atomising pressure:	200 KPa
Inlet air temperature:	143 - 160°C
Outlet air temperature:	70 - 95°C
Throughput:	Approximately 30 ml/min.
Estimated evaporation capacity:	1.5 kg / hr

Viscosity of the feed was considerably lower than that of the isoelectric process product and it was possible to spraydry the solutions as is (i.e. at approximately 14% solids). However, as in the case of the isoelectric process product, progressive buildup of solid material occurred in the drying chamber, limiting the length of run possible and adversely affecting product yield.

5.8 RESULTS

5.8.1 Analysis of Finished Product.

Chemical analysis results for the concentrated products from the UF unit and the finished spraydried products are summarised in Table 21.

Table 21. Analysis of Product Produced in Repeat Runs Under Optimised Conditions.

		BATCH 1	BATCH 2	BATCH 3
Product ex UF unit	% Protein % Solids % Protein (dry basis)	12.84 13.99 91.8	13.20 14.40 91.7	12.83 14.05 91.3
Spraydried product.	% Protein % Solids % Protein (dry basis)	81.89 95.42 85.8	84.30 95.42 88.4	85.09 96.93 87.8

5.8.2. Calculation of Yields

A similar procedure was followed to that adopted for the isoelectric process samples.

Yields were calculated in a number of different ways:

$$\text{Dry solids yield (UF only)} = \frac{\text{Wt of product ex UF unit} \times \% \text{ solids of product ex UF unit}}{\text{Wt of extract} \times \% \text{ solids of extract}} \times 100\%$$

$$\text{Protein yield (UF only)} = \frac{\text{Wt of product ex UF unit} \times \% \text{ protein of product ex UF unit}}{\text{Wt of extract} \times \% \text{ protein of extract}} \times 100\%$$

$$\text{Dry solids yield (extraction/UF)} = \frac{\text{Wt of product ex UF unit} \times \% \text{ solids of product ex UF unit}}{\text{Wt of flakes in original extraction} \times \% \text{ solids of flakes}} \times 100\%$$

$$\text{Protein yield (extraction/UF)} = \frac{\text{Wt of product ex UF unit} \times \% \text{ protein of product ex UF unit}}{\text{Wt of flakes in original extraction} \times \% \text{ protein of flakes}} \times 100\%$$

$$\text{Dry solids yield (spraydrying)} = \frac{\text{Wt of dried product} \times \% \text{ solids of dried product}}{\text{Wt of spraydryer feed} \times \% \text{ solids of spraydryer feed}} \times 100\%$$

$$\text{Protein yield (spraydrying)} = \frac{\text{Wt of dried product} \times \% \text{ protein of dried product}}{\text{Wt of spraydryer feed} \times \% \text{ protein of spraydryer feed.}} \times 100\%$$

5.8.3 Yields

The percentage yields obtained using the above methods are summarised in Table 22.

Table 22. Yields for Ultrafiltration Process.

		BATCH 1	BATCH 2	BATCH 3
UF process only	Dry solids Protein	56.8 85.9	55.8 83.9	54.9 83.5
Extraction/ UF process	Dry solids Protein	29.4 54.3	28.0 51.7	28.6 52.6
Spraydrying	Dry solids Protein	51.1 48.1	50.9 49.0	80.0 76.6

5.9 DISCUSSION.

Yield figures for the extraction / UF processes are consistent for the three runs. Both the yields and the proportion of protein in the final products are slightly higher for the UF products than for the isoelectric process, indicating the economic potential of the UF process. Spraydrying yields were variable with reasonably good results obtained for Batch 3 but unsatisfactory results for batches 1 and 2, indicating the practical difficulties experienced with small scale spraydrying.

Analysis figures for protein on a dry basis in the finished spraydried product are slightly lower than those quoted in existing published studies. Lawhon et al (1979) quote figures of 92.3% and 91.8% dry basis protein respectively for samples obtained using two different samples of defatted soya flakes as raw material. No yield figures have been quoted in published studies, making yield comparisons impossible.

6. PRODUCTION OF SOYA PROTEIN ISOLATE USING SWOLLEN GEL TECHNOLOGY.

6.1 LITERATURE REVIEW

The use of polymer gels for extraction purposes was first reported some ten years ago (Cussler et al 1984). Certain acrylic based polymers were found to have temperature dependent properties of absorbing and releasing liquids. In addition these properties were found to be size selective with smaller molecules taken up into the gels but larger molecules excluded. Further investigations into these properties followed (Freitas & Cussler 1987, Gehrke et al 1986, Gehrke & Cussler 1989) and the feasibility of this technique for soya protein extraction was specifically studied and shown to have potential. Trank et al (1989) used as feed material a soluble extract of defatted soya flakes produced by extraction at pH 8.5. They then used a poly (N-isopropylacrylamide) gel to extract the protein components by a multiple stage process comprising gel swelling at 5 °C followed by spin separation of the gel and supernatant, washing of the gel, collapsing of the gel by heating it to 40 °C and re-utilisation of the gel for further extractions. Trank et al (1989) also claim to be able to produce a final product containing 17% solids and 96% protein on a dry basis by means of a three stage extraction process.

6.2 PREPARATION OF GEL.

The procedure followed in earlier studies was used as follows:

1. 7.92g N-isopropylacrylamide and 0.079g N-N methylenebisacrylamide (both ex Polysciences Inc, Warrington, USA) were dissolved in 100 ml distilled water.
2. The solution was cooled in an ice bath and sparged with nitrogen for 10 minutes.
3. After 5 minutes sparging, 0.005g of ammonium persulphate was added as an initiator. After a further 5 minutes sparging 0.005g of sodium metabisulphite was added to accelerate the reaction.
4. After the addition of the sodium metabisulphite, the reaction vessel was sealed and the solution was left to come up to room temperature (20 - 25°C) overnight.
5. The resulting gel was heated to 50°C and cut into small pieces using scissors. These were then swollen and collapsed several times by repeated heating and cooling in water.

6.3 EXPERIMENTAL PROCEDURE

The polyisopropylacrylamide gel produced by the above method shows water absorption properties at temperatures below 30°C. Above this temperature the bound

water is rapidly released from the gel. The following procedure was therefore followed for the extraction of soya protein:

1. The collapsed gel was added to a soya extract produced by the standard single stage solubilisation procedure.
2. The extract and gel were allowed to stand for 16 hours at 5 - 10°C.
3. The supernatant ('retentate') was decanted off and any excess liquid adhering to the swollen gel was removed by spinning the gel contained in a mesh bag in a rotating basket. For small scale samples a kitchen salad spinner was used for this purpose.
4. The gel was washed with chilled water to remove any further adhering supernatant, with the water being added back to the concentrated retentate. The quantity of water added was varied according to the degree of concentration required. Final extractions excluded the washing step in order to achieve greater concentration.
5. The gel was collapsed by heating to 50 - 60°C for 30 minutes.
6. The liquid absorbed in the gel was discarded and the collapsed gel used for a further extraction procedure.

6.3.1 Effect of Temperature and Time on Liquid Absorption Rate by Gel.

A preliminary study was carried out in which the weight of water absorbed by the gel and degree of gel swelling were determined after immersion in excess water for different times at different temperatures. Results are shown in Table 23.

Table 23. Effect of Temperature on Degree of Gel Swelling.

Initial weight of collapsed gel: 25.4g Dry gel weight: 7.8g

	5°C		26°C	
Time (mins)	Gel weight (g)	Degree of swelling *	Gel weight (g)	Degree of swelling *
30	92.3	11.8	65.6	8.4
60	119.7	15.3	82.7	10.6
90	143.3	18.4	99.8	12.8
120	160.3	20.6	110.3	14.1

$$\text{* Degree of swelling} = \frac{\text{Gel weight}}{\text{Dry gel weight}}$$

The greater and more rapid absorption at the lower temperature is readily seen hence 5°C was adopted for subsequent extractions.

6.3.2. Preliminary Extraction of Soya Protein

A quantity of 24.7g of collapsed gel was added to 838.6g of standard soya extract. The extraction procedure listed above was carried out 3 consecutive times with samples of the concentrated retentate and absorbed liquid collected for analysis at each stage. Gel swelling was carried out overnight in each case. Results for the analysis of the samples are shown in Table 24.

Table 24. Analysis of Samples from 3 Stage Extraction Process.

		% Protein	% Solids	% Protein (dry basis)
	Initial extraction	2.56	4.42	57.9
1st Extraction	Retentate	3.12	5.02	62.2
	Absorbed liquid	1.02	2.78	36.7
2nd Extraction	Retentate	4.49	6.53	68.8
	Absorbed liquid	1.09	2.82	38.7
3rd Extraction	Retentate	6.84	9.15	74.8
	Absorbed liquid	1.73	3.64	47.5

Separation efficiency for each of the three stages was calculated for both solids and protein according to the method used in previous studies (Trank et al 1989).

$$\% \text{ separation} = \frac{\text{Actual Concentration Increase Obtained}}{\text{Theoretical Concentration Increase Based on Volume Change}}$$

As an example of the procedure, consider the solids separation for the first extraction:

Initial extract volume	=	838.6 ml
Initial extract solids	=	4.42%
Retentate volume	=	603.8 ml
Retentate solids	=	5.02%

Theoretical concentration increase based on volume change

$$= \frac{838.6}{603.8} = 1.389$$

Actual concentration increase

$$= \frac{5.02}{4.42} = 1.136$$

% separation efficiency (solids)

$$= \frac{1.136}{1.389} = 81.8\%$$

The effect of feed concentration on protein and solids separation efficiencies and degree of gel swelling is summarised in Table 25.

Table 25. Effect of Feed Concentration on Separation Efficiencies.

	% Protein Concentration in Feed	% Separation Efficiency (solids)	% Separation Efficiency (protein)	Degree of Swelling of Gel.
1st Extraction	2.56	81.8	87.6	32.6
2nd Extraction	3.12	78.0	86.2	31.4
3rd Extraction	4.49	75.7	82.2	20.0
Overall	-	48.3	55.3	-

Figures for protein separation efficiency and degree of gel swelling are similar to those observed by Trank et al for the same range of protein concentrations. It can be seen however that separation efficiencies for total solids are only slightly lower than those for protein, indicating that only limited separation of protein from non-protein components is taking place. A high degree of separation of protein from non-protein components (with non-protein components therefore removed from the retentate) would substantially reduce the solids content of the retentate, thereby reducing the actual concentration increase obtained and thus also the solids separation efficiency to a figure well below that of the protein separation efficiency. In practice only a small differential is observed between the two separation efficiencies for all three extractions. Further confirmation of poor separation efficiency is obtained from the relatively small increase in protein content on a dry solids basis for the retentate.

It is however evident from the above figures that it was not possible to achieve a retentate containing 17% solids and 96% protein on a dry basis by a three step process as is claimed by Trank et al.

Furthermore it can be seen that substantial quantities of protein are being lost in the liquid absorbed by the gel, even after washing of the gel, severely affecting the overall yield.

As a result of this, it was decided to carry out further extractions using a higher ratio of gel to extract in an attempt to improve yield and increase the proportion of protein in the final retentate solids. For all subsequent extractions, the complicated and confusing method used by Trank et al for calculating separation efficiencies was abandoned and a simple mass balance based on analysis of the initial and final products of the multi-extraction process was used to calculate yields in terms of percentage solids and percentage protein recovered. Analysis was also carried out in some cases on the absorbed liquid discarded at each extraction in order to obtain an indication of the protein losses at each stage.

6.3.3 Extraction at Higher Gel/Extract Ratio

A quantity of 121.0g of collapsed gel was added to 800g of standard soya extract. 3 consecutive extraction procedures were carried out and the initial and final products were analyzed to determine yield. Results are shown in Table 26.

Table 26. Analysis of Samples from Extraction Process at Higher Gel/Extract Ratio.

	% Protein	% Solids	% Protein (dry basis)
Initial extract	2.59	4.48	57.8
Final retentate	6.62	8.33	79.5

$$\% \text{ yield solids} = \frac{\text{Wt of final retentate (172.2) x \% solids final retentate (8.33)}}{\text{Wt of initial extract (800) x \% solids initial extract (4.48)}} \times 100\%$$

$= 40.0\%$

$$\% \text{ yield protein} = \frac{\text{Wt of final retentate (172.2) x \% protein final retentate (6.62)}}{\text{Wt of initial extract (800) x \% protein initial extract (2.59)}} \times 100\%$$

$= 57.3\%$

It can be seen that the efficiency of separation of protein from non protein components was improved (as observed by the increase in protein on a dry solids basis relative to that obtained in 6.3.2.).

Yields for 6.3.2 were calculated and found to be :

% yield (solids) = 40.8%

% yield (protein) = 52.6%

This gave a further indication of the improved efficiency of 6.3.3 however it was evident that considerable loss of protein was still occurring. The use of antifoam agent (Dow Corning 1510 silicone) was therefore investigated as this had been found to enhance yields in previously reported work (Trank et al 1988).

6.3.4 Extraction Using Anti Foaming Agent.

A quantity of 148.3g of collapsed gel was added to 1310g of standard soya extract (the need to subsequently split the batch into 2 portions necessitated a larger quantity of extract). The initial gel swelling process at 5°C was carried out overnight, after which the mixture was split into two portions. 0.1% of antifoam agent was added to one portion after which the extraction procedure was continued for both portions separately. 3 extractions were carried out for each portion and efficiency of protein separation and recovery with and without antifoam were assessed by analysis of the absorbed liquid and of the final product of each extraction sequence. Results are shown in Table 27.

Table 27. Effect of Anti Foaming Agent on Protein Extraction.

		% Protein	% Solids	% Protein (dry basis)
Initial Extract		2.67	4.48	59.6
1st Extraction	**Absorbed liquid with antifoam**	0.63	1.77	35.6
	Absorbed liquid without antifoam	0.62	1.90	32.8
2nd Extraction	**Absorbed liquid with antifoam**	0.58	1.18	49.2
	Absorbed liquid without antifoam	0.52	1.05	49.5
3rd Extraction	**Absorbed liquid with antifoam**	1.37	2.05	66.8
	Absorbed liquid without antifoam	1.33	2.01	66.2
Final retentate with antifoam		6.17	8.03	76.8
Final retentate without antifoam.		5.96	7.55	78.9

It was observed that no consistent improvement in protein extraction (as expressed by lower protein contents in the absorbed liquid) was observed in the samples containing antifoaming agent. Overall yields were therefore determined by combining the results of both sets of extractions and found to be:

% yield (solids) = 32.2%

% yield (protein) = 42.2%

These yields cannot be compared directly to those from previous experiments as a different gel/extract ratio had been used. However it was observed that a progressive increase in the liquid retained by the gel after collapsing was occurring after successive extractions as can be seen from the weight of the gel after collapsing (Table 28).

Table 28. Effect of Successive Extractions on Liquid Retention by Gel.

	No. of extractions	Gel weight after collapsing (g)	Wt of absorbed liquid lost during collapsing (g)
Initial Wt.		121.0	-
1st Experiment (6.3.3)	1	130.8	377.0
	2	141.2	345.0
	3	157.5	191.4
2nd Experiment (6.3.4)	4	201.2	794.7
	5	271.6	666.4
	6	367.7	569.7

This increase in bound water progressively reduces the absorption capacity of the gel, thus reducing the efficiency of protein separation.

6.3.5 Extraction Using Gel Regenerated by Vacuum Drying

In an attempt to overcome the problems caused by the progressive loss of gel absorption capacity, the gel used for the preceding experiments was vacuum dried overnight at 70°C. Gel weight was reduced from 367.7g to 100.6g. The dried gel was added to 1310g of standard soya extract and the extraction procedure was repeated, using 5 extractions instead of 3 in an attempt to further concentrate the protein and increase the solids content of the dried product. Analysis of the absorbed liquids and the final product gave the results shown in Table 29.

Table 29. Effect of Use of Vacuum Dried Gel on Protein Extraction.

	% Protein	% Solids	% Protein (dry basis)
Initial extract	2.78	4.57	60.8
1st extraction absorbed liquid	0.65	1.88	34.4
2nd extraction absorbed liquid	0.65	1.54	42.2
3rd extraction absorbed liquid	0.56	1.27	44.1
4th extraction absorbed liquid	0.93	1.59	58.5
5th extraction absorbed liquid	0.72	1.10	64.5
Final retentate	8.81	11.13	79.2
c.f. Average figures for final retentate from previous 3 stage process	6.07	7.79	77.9

Protein content expressed on a dry solids basis only increased very slightly as a result of the two additional extractions. The additional extractions however considerably reduced the overall yields, as did the higher solids content of the final product (due to the higher solution viscosity and consequent greater difficult in separating gel from solution).

The yields achieved were as follows:

% solids recovered = 17.5%

% protein recovered = 22.8%

Vacuum drying of the gel also had little effect on improving the absorption capacity of the gel as can be seen from Table 30.

Table 30. Effect of Vacuum Drying of Gel on Liquid Retention by Gel.

No. of extractions	Gel wt. after collapsing (g)	Wt. of absorbed liquid lost during collapsing (g)
Initial weight	100.6	-
1	398.8	482.4
2	484.2	475.0
3	517.3	386.1
4	555.6	365.2
5	509.1	370.2

It was observed that all the water lost during the vacuum drying was immediately taken up as bound water after the first extraction with the dried gel.

6.4 DISCUSSION OF PRELIMINARY FINDINGS

It can be concluded from these results that high absorption levels of liquid by the gel are only obtained for the first few extractions using a fresh batch of gel, after which progressive deterioration in absorption capacity occurs, with the gel absorbing less than its own weight of liquid after less than 10 extraction cycles. This places severe limitations on the feasibility of the process in view of the need to use large quantities of gel to obtain an acceptable separation and the very high cost of the gel (approximately R8000/kg). It was also apparent that in terms of both protein content on a dry basis of the end product and product yield, the gel extraction process compared very unfavourably to both the isoelectric and ultrafiltration processes.

A further side effect of the deterioration of gel absorption capacity was the increase in soluble solids retained in the gel after collapsing. This resulted in microbiological spoilage of the gel which in turn affected the extraction solutions. Microbial spoilage of the final retentates was such that their pH's dropped from approximately 8.5 in the initial extract to levels where isoelectric precipitation of the protein occurred.

In view of all these factors, it was decided that production of soya protein isolate in quantities sufficient for spraydrying and evaluation of functional properties was not a practical proposition. Instead, a small scale extraction was carried out in triplicate to produce samples in liquid form for analysis purposes only.

6.5 EXTRACTIONS USING STANDARDISED PROCEDURE.

The following standard procedure was adopted:

1. Add 73g freshly produced gel to 950ml standard soya extract.
2. Chill overnight at 5°C.
3. Separate gel and gel retentate by spinning and wash gel with 400ml chilled water.
4. Combine gel retentate and wash water.
5. Collapse gel, discard absorbate and add collapsed gel to mixture from (4)
6. Repeat steps (2) - (5).
7. Repeat steps (2) and (3) and collect final concentrated retentate.

The above procedure was carried out in triplicate, using the same batch of soya extract as feed material for all three series of extractions.

6.6 RESULTS

6.6.1 Analysis of Finished Product

As explained in 6.4, chemical analysis of the final concentrated retentates only was carried out. Results are shown in Table 31.

Table 31. Analysis of Retentates from Repeat Standard Extraction Procedure.

	Batch 1	Batch 2	Batch 3
% Protein	9.36	7.75	7.80
% Solids	11.71	9.48	9.58
% Protein (dry basis)	79.9	81.8	81.4

6.6.2 Calculation of Yields

Yields for the separation process only were calculated as a standard extract was used for all three batches. Yields were calculated as follows:

$$\text{Dry solids yield} = \frac{\text{Wt of final retentate x \% solids of final retentate}}{\text{Wt of initial extract x \% solids of initial extract}} \times 100\%$$

$$\text{Protein yield} = \frac{\text{Wt of final retentate x \% protein of final retentate}}{\text{Wt of initial extract x \% protein of initial extract}} \times 100\%$$

6.6.3 Yields

Using the above methods, percentage yields were obtained for the separation process. Results are shown in Table 32.

Table 32. Yields from Repeated Standard Extraction Procedure.

	Batch 1	Batch 2	Batch 3
Dry solids yield	18.6	31.3	31.5
Protein yield	25.1	43.2	43.3

6.7 DISCUSSION

In spite of using identical batch sizes and gel/extract ratios for all three extractions, it can be seen that Batch 1 differed considerably from Batch 2 and Batch 3. The higher final % solids content was achieved in Batch 1 at the expense of both yield and protein content on a dry basis. The difference cannot readily be explained but serves to further indicate the erratic nature of the gel extraction process. In any event, yields for all three extractions are substantially lower than those for both the isoelectric and ultrafiltration processes. No comparative yield figures are available for the studies of Trank et al (1989). However the results obtained cast further doubt over their claim to be able to obtain an extract with 17% solids and 96% protein on a dry basis by a three stage procedure.

Samples of the final retentates were deep frozen and stored for subsequent analysis.

7. COMPARISON OF THREE PROPOSED PROCESSES.

It is relevant at this stage to compare results from the three processes. Average figures for product composition and yield are summarised in Table 33.

Table 33. Comparison of Finished Spraydried Products and Yields for Samples from Isoelectric, Ultrafiltration and Swollen Gel Processes.

	□ Isoelectric process	□ UF process	□ Swollen gel process
Finished product:			
% Protein	77.4 ± 0.6	83.8 ± 1.4	Liquid product only produced
% Solids	91.1 ± 1.4	95.9 ± 0.7	
% Protein (dry basis)	84.9 ± 1.4	87.3 ± 1.1	81.0 ± 0.8
Yield* (%):			
Solids	52.9 ± 1.8	55.8 ± 0.8	27.1 ± 6.0
Protein	81.7 ± 2.6	84.4 ± 1.0	37.2 ± 8.6

* Yield figures refer only to the separation stage (i.e. initial extraction yields and drying yields are not included).

□ Average figures for three standardised runs using each process.

The performance of each process can be judged in terms of:

i. the efficiency of separation of protein from non-protein components as expressed by the percentage protein on a dry basis in the finished product

ii. the yields of solids and protein obtained at the separation stage, bearing in mind that the initial extraction and final drying stages are common to all three processes.

It can be seen that the ultrafiltration process shows a small but significant advantage over the isoelectric process in all respects. The swollen gel process compares unfavourably to both the ultrafiltration and isoelectric processes, particularly in terms of yield.

The ultrafiltration process also appears to be the most consistent of the three processes in view of the lower standard deviations obtained for yield figures for the UF process. In this respect the erratic nature of the swollen gel process is further emphasised, even though the finished product from this process is, in terms of standard deviation for percentage protein on a dry basis, the most consistent of the three.

8. EVALUATION OF FUNCTIONAL PROPERTIES OF SOYA PROTEIN ISOLATES.

8.1 INTRODUCTION & LITERATURE REVIEW

Much of the commercial value of soya protein lies in their functional properties rather than as a protein source and their benefits in this respect have been extensively reviewed (Johnson 1970, Kinsella 1979, Mattill 1971, Smith & Circle 1977) Very extensive studies have been carried out on the various functional properties, and a wide range of methodologies for quantitative measurement have been devised (Hermansson 1979). Most of these methods are suitable for comparative purposes only and make use of either 'artifical systems' (in which a simple system containing readily measurable parameters is used) or 'model systems' (in which the characteristics of a typical food product are simulated) to obtain data. Artificial systems have rightly been criticised (Puski 1976) for their lack of relevance to true process conditions but are of value if the comparative data obtained from them can be correlated with actual performance in food systems. Model systems are theoretically of greater value but comparative results may be influenced by variations in the raw materials used to produce the model system. The methods may also require the use of fairly large sample sizes.

8.2 FUNCTIONAL PROPERTIES STUDIED.

For the purposes of this study the following functional properties were quantitatively measured:

8.2.1 Solubility

This is the single most important property of the isolate and has a major influence on the full range of functional properties (Kinsella 1979, Shen 1976). Solubility of proteins is normally expressed in terms of either Protein Dispersibility Index (PDI) or Nitrogen Solubility Index (NSI). Both of these are determined by dispersing the protein in water (using a high speed / short time method for PDI and lower speed / longer time for NSI) followed by centrifugation of the mixture and analysis of the resulting supernatant liquid for protein content, which is then compared to the protein content of the original dry material. Standard methods for both PDI and NSI are well established (AOCS 1989). Determination of NSI for the isolates used in this study was impractical due to the lumping and insufficient dispersion of material occurring at the low stirring speeds required, hence a slightly modified verson of the AOCS method for PDI was used to determine solubility characteristics.

8.2.2 Water Absorption Characteristics.

The ability of soya proteins to absorb and bind water is of particular importance in meat products where their use helps to prevent shrinkage of processed meat products during cooking, thus enhancing juiciness and palatability of the product. Water absorption properties hve been studied by two different methodologies.

a) Dispension/centrifugation methods where the volume of water retained by the isolate after centrifugation is measured (Ashraf & Lee 1988, Beuchat 1977, Hutton & Campbell 1977)

b) The so-called Baumann apparatus in which the spontaneous takeup of water by isolates under non-agitated conditions is determined (Arrese et al 1991,

Torgersen & Toledo 1977, Sorgentini et al 1991).

The Baumann apparatus comprises a filter paper mounted in a funnel connected to a graduated pipette. The apparatus is filled with water and, when the isolate is sprinkled on to the filter paper, the amount of water absorbed is measured by movement of the water meniscus in the pipette. This method was initially evaluated with a view to using it for this study but proved to be extremely time consuming and gave poor repeatability of results. A dispersion / centrifugation method was therefore adopted which had the further advantage of using the same sample used for PDI determinations.

8.2.3 Oil Absorption Characteristics.

These are also of particular value in meat products and similar consideration to those for water absorption characteristics apply. Both dispersion / centrifugation methods (Ashraf & Lee 1988, Beuchat 1977, Manak et al 1980) and the Baumann apparatus (Elizalde et al 1991, Kanterewicz et al 1987) have been used to study oil absorption characteristics. For the purposes of this study a dispersion / centrifugation method similar to that used for water absorption measurements was adopted.

8.2.4 Emulsifying Properties.

These are again primarily of importance in meat systems but are also of value in such products as baked goods and coffee whiteners. Both emulsion capacity and emulsion stability properties have been extensively studied. Emulsion capacity methods involve the progressive addition of oil to an aequeous slurry of the isolate and determination

of the breakdown point of the resulting emulsion. They have however been severely criticised (Puski 1976) as being of very little relevance to actual food systems and their value, even for comparative purposes, is highly debatable. Emulsion stability determinations involve preparation of an emulsion and measurement of the volumes of separated liquid after either centrifugation (Ashraf & Lee 1988, Franzen & Kinsella 1976, Inklaar & Fortuin 1969) or prolonged standing (Titus et al 1968, Tornberg & Hermansson 1977).

It was decided to confine the study of emulsifying properties to measurement of emulsion stability only. The method of Inklaar & Fortuin (1969) has been particularly extensively used and it was adopted with certain minor modifications for the purposes of this study.

8.2.5 Viscosity

The viscosity generating properties of the more highly soluble isolates are well known. Measurements are easily made using a standard viscometer and for this study a Brookfield viscometer was used.

8.2.6 Gelling Properties

Certain soya isolates display gel forming properties when their aequeous solutions are heated in the range 70 - 90°C. Properties of the gels such as melting point (Babajimopoulos et al 1983, Catsimpolas & Meyer 1970), freeze / thaw stability (Ehninger & Pratt 1974), viscosity (Fiora et al 1990, Puski 1975) and gel strength (Manak et al 1980, Sherner et al 1978, Torgersen & Toledo 1977, Utsumi & Kinsella

1985) have been studied. However, as in the case of emulsion capacity, the relevance of these properties to actual food systems is questionable and, in addition, the samples produced for this study showed only very limited and erratic gelling properties. It was therefore decided not to study gelling properties.

8.2.7 Foaming Properties.

Certain soya proteins have the ability to form and stabilise a foam when their aqueous dispersions are whipped and stirred. These properties are particularly valuable in products such as instant desserts and aerated sugar confectionery. Foaming capacity can readily be determined by standardised whipping procedures and the measurement of parameters such as changes in density (Chen & Morr 1985), overrun (German et al 1985) and volume increase (Lawhon et al 1972, McWatters & Cherry 1977). Foam stability has been studied by measuring quantities of liquid lost on standing (Chen & Morr 1985, German et al 1985) and loss of volume on standing (Franzen & Kinsella 1976, Puski 1976). For this study, foam density and quantities of liquid on standing were determined, using a procedure considered to be reasonably representative of actual process conditions.

8.3 METHODS

8.3.1. Method for Determination of PDI-Protein Dispersibility Index

1. Place 300ml of water at 25°C in a 600ml beaker.

2. Immerse the mixing head of a Silverson L4R laboratory mixer in the water.

3. While running the mixer at low speed, rapidly pour in 20g of the isolate sample.

4. Increase the mixer speed to setting 4 and mix / disperse for 10 minutes. Scraping of the vessel sides and mixing head may be necessary initially to prevent the formation of lumps.

5. Stop the mixer and allow the mixture to stand until any foam formed has dispersed sufficiently for a 150g sample of non-aerated mix to be removed.

6. Weigh a 150 $\pm$ 0.2g sample of the mixture into a suitable centrifuge tube.

7. Centrifuge the mixture for 10 minutes at 2000 rpm.

8. Fully decant off the supernatant liquid and filter it through a glass wool plug.

9. Determine the protein content of 5 ml aliquots of the filtered supernatant by the Kjeldahl method.

10. Calculation of PDI:

Slurry concentration : 20g isolate in 300 ml water

∴ Soluble protein of 1g of isolate is contained in 15 ml of supernant

$$\therefore \text{PDI} = \frac{\text{Kjeldahl titre} \times \text{normality of titrating acid} \times 1.4 \times 6.25 \times \frac{15}{5}}{\text{\% protein of isolate as is}} \times 100\%$$

8.3.2 Method for Determination of Water Absorption Capacity - WAC.

Steps 1 to 8 - as for determination of PDI - the same sample is used.

9. Weigh accurately the quantity of supernatant liquid removed (before filtration for PDI determination).

10. Calculation of WAC:

$$\text{WAC} = \frac{\text{Wt of sample centrifuged} - \text{Wt of supernatant decanted}}{\text{Wt of sample centrigued} \times \frac{20}{300}}$$

8.3.3 Method for Determination of Oil Absorption Capacity - OAC.

1. Place 300 ml of sunflower oil at 25°C in a 600 ml beaker.

2. Immerse the mixing head of a Silverson L4R laboratory mixer in the oil.

3. While running the mixer at low speed, rapidly pour in 20g of the isolate sample.

4. Increase the mixer speed to setting 4 and mix / disperse for 10 minutes. Scraping of the vessel sides and mixing head may be necessary initially to prevent the formation of lumps.

5. Stop the mixer and accurately weigh a 150 $\pm$ 0.2g sample of the mixture into a suitable centrifuge tube.

6. Centrifuge the mixture for 10 minutes at 2000 rpm.

7. Decant off the supernatant oil (N.B. a layer of semi solid sludge may also form at the top of the centrifuge tube and care must be taken to retain this in the tube while pouring off the supernatant).

8. Weigh accurately the quantity of supernatant oil removed.

9. Calculation of OAC :

$$\text{OAC} = \frac{\text{Wt of sample centrifuged - Wt of supernatant decanted}}{\text{Wt of sample centrifuged} \times \frac{20}{300}}$$

8.3.4. Method for Determination of Emulsion Stability

1. Place 180ml of water and 100ml of sunflower oil at 25°C in a 600ml beaker.

2. Immerse the mixing head of a Silverson L4R laboratory mixer in the liquid.

3. While running the mixer at low speed, rapidly pour in 10g of the isolate sample.

4. Increase the mixer speed to setting 4 and mix / disperse for 10 minutes. Scraping of the vessel sides and mixing head may be necessary initially to prevent the formation of lumps.

5. After 10 minutes mixing, transfer the beaker and contents to a waterbath set at 85°C. Heat the beaker for 15 minutes with occasional gentle stirring.

6. Cool the beaker and contents in water (20 - 25°C) for 15 minutes.

7. Accurately weigh a 150 ± 0.2g sample of the mixture into a suitable centrifuge tube.

8. Centrifuge the mixture for 10 minutes at 2000 rpm.

9. Determine the volume of the upper layer formed after centrifugation.

10. Emulsion stability = 150 - volume of top layer.

8.3.5 Method for Determination of Foaming Properties.

1. Blend 110g icing sugar and 5g isolate in a stainless steel laboratory Hobart mixer bowl.

2. Add 70ml of water at 25°C.

3. Whip the mixture for 6 minutes using a Hobart laboratory mixer at maximum speed with an N5D cage wire beater.

4. Pour the resulting foam into a tared 100ml measuring cylinder and determine the weight of 100ml of foam.

5. $$\text{Foaming capacity} = \frac{1}{\text{specific gravity of foam.}}$$

6. The foam is then allowed to stand for 18 hours at room temperature.

7. After 18 hours the volume of liquid separated from the foam is determined.

8. Foam stability = 100 - vol of liquid separated.

8.3.6 Method for Determination of Viscosity

1. Prepare a 10% (w/w) dispersion of the isolate in water at 25°C by mixing for 10 minutes using a Silversen L4R laboratory mixer at speed 4.

2. Pour 150ml of the dispersion into a 250ml beaker and determine its viscosity using a Brookfield HAT viscometer. 4 consecutive readings are taken for each sample.

8.4 RESULTS

Measurements of the various functional properties were carried out in duplicate for samples from each of the three standardised batches produced by the isoelectric and ultrafiltration process, thus giving a total of six determinations for product from each process.

The results for the experimental samples were compared with those obtained for a series of commercially available soya isolates, namely:

PP500E - an isolate for use in meat products with high water absorption and emulsification capabilities.

Supro 670 - an isolate with added lecithin and consequent high dispersibility, normally used as a protein supplement.

Supro 710 - an isolate with strong emulsifying capability, used in coffee creamers and whipped toppings.

Ardex F - an isolate with limited functionality, used primarily for protein fortification.

Functional properties were measured in duplicate for each of the four commercial soya isolates.

8.4.1 Results for Protein Dispersibility Index (PDI).

These are shown in Table 34.

Table 34. PDI's of Experimental and Commercial Soya Isolates.

SAMPLE	PDI (%)
Isolectric process samples	79.0 ± 1.3
Ultrafiltration process samples.	91.8 ± 0.6
PP500E	68.8 ± 4.5
Supro 670	70.0 ± 0.8
Supro 710	89.4 ± 0.2
Ardex F	37.5 ± 1.0

8.4.2 Results for Water Absorption Capacity (WAC)

These are shown in Table 35.

Table 35. Water Absorption Capacities of Experimental and Commercial Soya Isolates.

SAMPLE	WAC
Isoelectric process samples	2.76 ± 0.32
Ultrafiltration process samples	2.25 ± 0.27
PP500E	6.56 ± 0.32
Supro 670	3.19 ± 0.14
Supro 710	0.46 ± 0.01
Ardex F	7.12 ± 0.08

8.4.3. Results for Oil Absorption Capacity (OAC)

These are shown in Table 36.

Table 36. Oil Absorption Capacities of Experimental and Commercial Soya Isolates.

SAMPLE	OAC
Isoelectric process samples	2.12 ± 0.08
Ultrafiltration process samples	2.35 ± 0.04
PP500E	2.55 ± 0.04
Supro 670	2.37 ± 0
Supro 710	2.86 ± 0.02
Ardex F	2.56 ± 0.17

8.4.4 Results for Emulsion Stability (ES)

These are shown in Table 37.

Table 37. Emulsion Stabilities of Experimental and Commercial Soya Isolates.

SAMPLE	EMULSION STABILITY
Isoelectric process samples	78.7 ± 3.9
Ultrafiltration process samples	86.6 ± 1.8
PP500E	84.3 ± 1.6
Supro 670	91.8 ± 0.1
Supro 710	93.9 ± 0.1
Ardex F	86.5 ± 0.4

8.4.5. Results for Foaming Properties

These are shown in Table 38.

Table 38. Foaming Properties of Experimental and Commercial Soya Isolates.

SAMPLE	FOAMING CAPACITY	FOAM STABILITY
Isoelectric process samples	1.19 ± 0.04	48.2 ± 1.6
Ultrafiltration process samples	0.97 ± 0.01	30.8 ± 2.3
PP500E	0.89 ± 0.01	33.5 ± 0.5
Supro 670	1.60 ± 0.01	60.0 ± 1.0
Supro 710	3.41 ± 0.03	84.5 ± 0.5
Ardex F	0.82 ± 0.01	18.5 ± 0.5

8.4.6. Results for Viscosity

These are shown in Table 39.

Table 39. Viscosities of Experimental and Commercial Soya Isolates.

SAMPLE	VISCOSITY (CPS)
Isoelectric process samples	143 ± 25
Ultrafiltration process samples	75 ± 9
PP500E	413 ± 11
Supro 670	46 ± 1
Supro 710	19 ± 1
Ardex F	563 ± 5

8.5 DISCUSSION

The ultrafiltration process samples gave the highest PDI values with only one of the commercial samples (Supro 710) giving a comparable value. WAC values were slightly higher for the isoelectric process samples than for the ultrafiltration process samples, however both the PP500E and Ardex commercial products had WAC values substantially higher than any of the experimental samples.

There appears to be a negative correlation between PDI and WAC - the sample with the highest WAC (Ardex F) also had the lowest PDI value. This can be explained in terms of the methodology adopted - a highly soluble isolate with high PDI will leave only a small portion of insoluble material available for physical binding of water after the initial high- shear dispersion / solubilisation process used in the method. Existing studies on the effect of denaturation (and consequent loss in protein solubility) on water binding properties (Sorgentini et al 1991, Wagner & Anon 1990) have confirmed this effect. This implies that PDI can be used as an inversely proportional measure of the water binding properties of soya isolates.

Figures for OAC show far less variation than those for WAC. The ultrafiltration process samples were slighly higher in OAC than the isoelectric process samples with the commercial samples showing no clear pattern other than Supro 710 which showed the highest OAC value along with the lowest WAC value.

The ultrafiltration process samples also had higher ES values than the isoelectric process samples however both Supro 670 and Supro 710 had higher ES values than any of the experimental samples.

Foaming properties of both isoelectric and ultrafiltration process samples were poor. Supro 710 however showed extremely good foaming properties and it is surprising that this particular product is not marketed as a potential whipping agent.

Viscosity of the ultrafiltration process samples is lower than that of the isoelectric process samples. Considerable variation in viscosity was observed for the commercial samples with Supro 710 and Supro 670 showing lower viscosity and PP500E and Ardex F higher viscosity than any of the experimented samples.

In overall terms, the result confirmed that formal measurement of functional properties is likely to be of only limited practical value in determining the likely applicability of a particular isolate for a specific purpose (other than foaming properties, where a clear cut result was obtained). The results are of value for comparison purposes only. Informal discussions with Protein Technologies International indicated that formal measurement of functional properties of soya isolates is rarely undertaken, with most evaluations carried out by incorporating the different isolates in the actual food system concerned. This is surprising in view of the very sizeable number of studies published in this field and it suggests the need for greater awareness by researchers of the practical implications of their methodology and findings in this field.

9. EVALUATION OF ANTI-NUTRITIONAL FACTORS OF SOYA PROTEIN ISOLATES.

9.1 TRYPSIN INHIBITOR ACTIVITY (T.I.A.)

9.1.1 Literature Review

The classic studies of Osborne & Mendel (1917) showed that growth in rats fed soybeans was only promoted if the beans had been subjected to heat treatment. Subsequent studies (Kunitz 1945) demonstrated the existence of a heat labile trypsin inhibitor in soybeans. Investigations into the mechanism of action of the trypsin inhibitor (Chernick et al 1948) indicated that this involved hypertrophy of the pancreas which in turn caused secretion by the pancreas of essential sulphur-containing amino acids with consequent drain on the body tissue of these amino acids and their resulting unavailability for growth purposes.

Very extensive work has subsequently been carried out on optimising conditions for trypsin inhibitor destruction during processing of soya proteins (Smith & Circle 1977).

Specific studies were carried out on isoelectric process isolates (Rackis 1966, Honig et al 1987) where trypsin inhibitor levels were found to be dependent on the degree of heat treatment to which the raw materials had been submitted and on the pH used for isoelectric precipitation. Trypsin inhibitor levels in ultrafiltration process

isolates have also been studied (Omosaiye & Cheryan 1979) and found to be fairly high, possibly due to complexing of the relatively low molecular weight trypsin inhibiting protein fractions with higher molecular weight proteins.

Methodology for quantitative determination of trypsin inhibitor activity involves the use of the synthetic substrate benzoyl - DL - arginine - p - nitroanilide (BAPNA) whose decomposition products when reacted with trypsin can be estimated colorimetrically. The addition of materials with T.I.A. to the reaction mixture reduces the degree of colour generation and enables T.I.A. to be determined by comparison with a reference standard containing no T.I.A. This method was originally devised by Kakade et al (1969). The method was further refined by Kakade et al (1974) by improving sample preparation procedures. Smith et al (1980) made further minor adjustments to the method, making it more suitable for handling large numbers of samples. The method of Smith et al (1980) was consequently adopted for this study.

9.1.2 Method

The method used was that of Smith et al (1980).

REAGENTS

i. Tris buffer. Dissolve 6.05g tris hydroxymethyl methylamine and 2.84g calcium chloride dihydrate in 900ml distilled water. Adjust pH to 8.2 with hydrochloric acid and make up to 1 litre.

ii. BAPNA substrate. Dissolve 40mg benzoyl-DL-arginine-p-nitroanilide hydrochloride (Sigma Chemical Company) in 1ml dimethyl sulphoxide and

dilute to 100 ml with tris buffer previously warmed to 37°C. Keep reagent at 37°C and prepare fresh reagent daily.

iii. Standard trypsin solution. Dissolve 40mg crystalline bovine trypsin (activity 10 units/mg) (Sigma Chemical Company) in 0.001M hydrochloric acid and make up to 2 litres with the acid. Solution is stable for 2 weeks at 4°C.

PROCEDURE

i. Grind dry samples finely to pass through 100 mesh sieve. Wet samples are blended to give a smooth paste.

ii. Shake a sample equivalent to approximately 1g dry product with 50 ml 0.01M sodium hydroxide. Use a high shear mixer (Silverson) to disperse the sample if necessary.

iii. Adjust pH of mixture to 9.4 - 9.6 using hydrochloric acid or sodium hydroxide solution. Leave to stand overnight at 4°C.

iv. Dilute the sample as required. Several determinations may be required to achieve the desired dilution.

v. Prepare the following mixtures in 10ml tubes.

[a]	Reagent blank	-	2ml distilled water.
[b]	Trypsin standard	-	2ml distilled water + 2ml trypsin solution.
[c]	Sample blanks	-	1ml diluted sample extract + 1ml distilled water.
[d]	Samples	-	1ml diluted sample extract + 1ml distilled water + 2ml trypsin solution.

vi. Mix contents of tubes and place in 37°C water bath for 10 mins.

vii. Add 5 ml BAPNA solution (warmed to 37°C) to each tube and incubate at 37°C for 10 minutes.

viii. Add 1ml of 30% acetic acid to each tube after exactly 10 minutes to stop the reaction.

ix. Add 2ml of standard trypsin solution to the reagent blank and sample blank tubes.

x. Filter the content of each tube through a 542 Whatman paper and measure the solution absorbance at 410 nm

Absorbance change due to trypsin inhibition per ml of sample extract.

$A_I = (A_b - A_a) - (A_d - A_c)$ where:

A_a = Reagent blank absorbance

A_b = Trypsin standard absorbance

A_c = Sample blank absorbance

A_d = Sample absorbance

$$\% \text{ inhibition} = \frac{A_I}{(A_b - A_a)} \times 100\%$$

% inhibition should be between 40% and 60%. If figures outside this range are found, dilution must be adjusted and a further determination carried out.

$$\text{Trypsin inhibitor activity (TIA)} = \frac{2.632 \times \text{dilution} \times A_I}{\text{sample wt.}} \text{ mg pure}$$

trypsin inhibited per g of sample

Determinations were carried out in duplicate on the defatted soya flakes, the

full range of experimental samples from the three processes and the four commercial products. As the swollen gel samples could only be analyzed in solubilised form (insufficient samples were available for spraydrying - see 6.4), trypsin inhibitor activity determinations were also carried out on samples of an isoelectric process slurry (i.e. the isoelectric product prior to spraydrying) and an ultrafiltration process concentrate (i.e. the ultrafiltration product prior to spraydrying). This enabled the effect of spraydrying on trypsin inhibitor content to be evaluated and a fair comparison to be made between the three sets of experimental samples.

In view of the adoption of a standardised single stage extraction process for the solubilisation of protein rather than the 'best process' 2 stage extraction originally devised, trypsin inhibitor activity was also determined on samples of 'standard' 1 stage extract and 'best process' 2 stage extract.

9.1.3. Results

Results are shown in Table 40. All results are expressed as mg pure trypsin inhibited per gram.

Table 40. Trypsin Inhibitor Activities of Experimental and Commercial Soya Isolates.

SAMPLE	T.I.A./g (dry sample)	T.I.A./g protein (dry basis)
Defatted soya flakes	19.3 ± 0.4	38.8 ± 0.8
Isoelectric process (dry product)	11.9 ± 0.7*	14.0 ± 0.8*
Isoelectric process (slurry before drying)	15.4 ± 0.3	17.7 ± 0.3
Ultrafiltration process (dry product)	25.6 ± 0.5*	29.3 ± 0.6*
Ultrafiltration process (concentrate before drying)	29.4 ± 0.5	32.1 ± 0.5
Swollen gel process (solubilised product)	4.3 ± 0.1*	5.3 ± 0.1*
Commercial soya isolates : PP500E	2.7 ± 0.1	3.1 ± 0.1
Supro 670	1.4 ± 0	1.5 ± 0
Supro 710	1.3 ± 0	1.4 ± 0
Ardex F	1.9 ± 0.1	2.1 ± 0.1
Standard extract (1 stage extraction)	32.5 ± 0.1	53.8 ± 0.2
Best process extract (2 stage extraction)	33.8 ± 0.5	54.5 ± 0.8

* Mean of 6 determinations (duplicate determination for each of 3 standard samples from each process).

All other results are mean of 2 determinations.

9.1.4. Discussion

The ultrafiltration process samples were found to have the highest T.I.A. values, confirming the previously reported unsuitability of the UF process in this respect (Omosaiye & Cheryan 1979). The isoelectric process samples showed intermediate levels of T.I.A. while the swollen gel samples had low T.I.A. values, indicating that the conditions under which the swollen gel extraction is carried out are not conducive to the protein complexing postulated for the UF process, thus enabling most of the trypsin inhibiting protein fraction to be lost in the gel absorbate. It can also be seen that the spraydrying process causes a slight further reduction in T.I.A. levels for the isoelectric and UF products.

All the commercial products however showed low levels of T.I.A., indicating that the processes used for their manufacture incorporate steps specifically aimed at reducing T.I.A..

The high levels of T.I.A. found in the experimental samples relative to those of the commercial samples give cause for concern. Potential reasons for the high T.I.A. values and ways of reducing these in the experimental isolates are discussed in Section 9.2.

Finally, only small differences were seen between T.I.A. values for the standard and best process extracts, indicating that the choice of extraction process would have little effect on final product T.I.A. values.

9.2 INVESTIGATION OF HIGH TRYPSIN INHIBITOR ACTIVITY IN EXPERIMENTAL SAMPLES.

The relatively high levels of trypsin inhibitor in the isoelectric and ultrafiltration process experimental samples gave cause for concern, hence further investigations were carried out.

9.2.1. Isoelectric Process Product.

It has been observed that, during isoelectric precipitation of soya protein, much of the trypsin inhibitor activity is removed in the whey (Rackis 1966). Later studies (Honig, Rackis & Wolf 1987) showed that the degree to which trypsin inhibitor activity is reduced is considerably affected by the pH used for precipitation, with a pH of 3.5 found to give maximum reduction. Due to the high residual trypsin inhibitor activity in the whey, thorough washing of the precipitated protein is also desirable.

The following procedure was followed to investigate the effects of pH and precipitate washing efficiency:

1. A sample of standard soya extract was prepared by the standard process (pH9.0 / 55°C / 15 min stirring).

2. The extract was split into 2 portions. One portion was adjusted to pH 4.25 and the other portion to pH 3.5.

3. Both portions were centrifuged, the whey decanted, then each of the resulting precipitates was further subdivided into 2 portions. One portion of each precipitate was washed once and the other portion three times with water.

4. Trypsin inhibitor activities were determined in duplicate for the four resulting slurries on a dry solids basis. Results are shown in Table 41.

Table 41. T.I.A. for Isoelectric Process Slurries Produced Using Varying pH's and Number of Washings.

SAMPLE			T.I.A. (mg pure trypsin inhibited /g dry solids)		
Slurry	pH 4.25	1 wash	12.5	±	0.3
Slurry	pH 4.25	3 washes	11.4	±	0.3
Slurry	pH 3.5	1 wash	5.7	±	0.1
Slurry	pH 3.5	3 washes	4.0	±	0.2

All results are mean of 2 determinations.

It can be seen that, while the improved washing efficiency reduces trypsin inhibitor activity to a limited extent, use of a lower pH for precipitation purposes has a much greater effect and gives a product which, after spraydrying, would have residual trypsin inhibitor activity approaching that of the commercially produced isolates. The increased numbers of washing stages could however adversely affect process yields.

9.2.2. Ultrafiltration Process Product.

The ultrafiltration process has been shown to give products high in trypsin inhibitor activity and it is suggested that this is due to protein / protein interactions preventing the removal of the low molecular weight trypsin inhibitor fractions of the protein (Omosaiye & Cheryan 1979).

It was therefore concluded that a reduction in trypsin inhibitor activity could only be achieved by high temperature / short time processing of the extract prior to ultrafiltration.

9.2.2.1 ***Steam Injection of Soya Extract***

This was carried out by passing the soya extract through the apparatus shown in Fig.7 (Barnes 1984).

The apparatus enables live steam to be injected into the extract, rapidly raising its temperature. A short holding time is followed by venting of the extract at the outlet which rapidly reduces its temperature. Two experiments were carried out using standard extract pumped through the apparatus at different throughputs and in each case trypsin inhibitor activity of the extract before and after treatment was determined. Results are shown in Table 42.

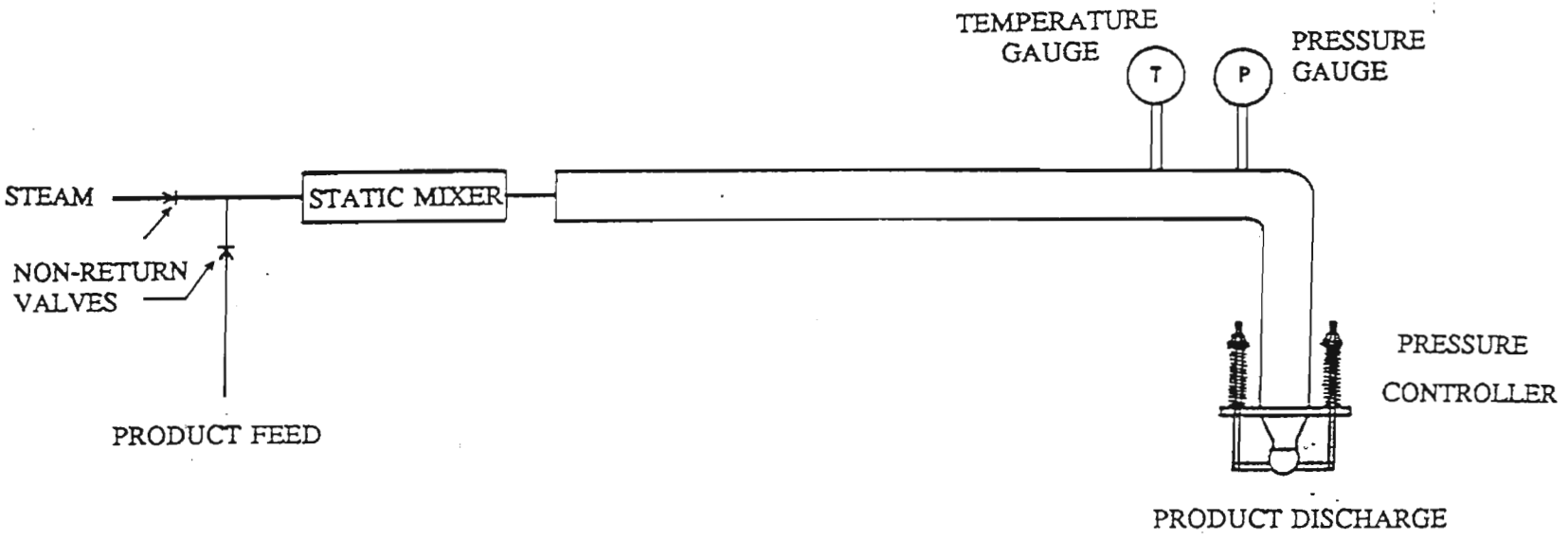

Fig. 7. Schematic Diagram of Steam Injection Apparatus [from Barnes (1984)]

Table 42. Effect of Steam Injection on T.I.A. of Extracts.

	Run 1	Run 2
Throughput (kg / min)	1.3	0.5
Steam pressure (KPa)	230	230
System pressure (KPa)	240-250	260
Temperature °C	132	130
% Solids (extract)	4.70	4.52
% Solids (treated extract)	4.54	4.05
T.I.A. (extract) (mg pure trypsin inhibited /g dry solids)	21.1	22.8
T.I.A. (treated extract) (mg. pure trypsin inhibited /g dry solids)	11.3	10.7

The process was shown to reduce the T.I.A. of the extract to approximately half its initial value. Although slightly better results were obtained at the lower throughput, the process was extremely slow and resulted in excessive dilution of the extract. It was decided to carry out a trial using an extract steam injected at the higher throughput, followed by ultrafiltration.

9.2.2.2 *Ultrafiltration of steam injected extract.*

A standard extract was steam injected as per run 1 above and then submitted to the standard ultrafiltration procedure. Analysis of the untreated and treated extracts and the final concentrate from the ultrafiltration process for trypsin inhibitor activity gave the results shown in Table 43.

Table 43. Effect of Steam Injection of Extract on T.I.A. of UF Process Product.

	T.I.A. (mg pure trypsin inhibited /g dry solids)
Extract as is	24.9
Steam injected extract	14.7
UF concentrated extract	15.1

The results confirmed that the ultrafiltration process has no effect on trypsin inhibitor activity. In addition yield figures for the extraction / steam injection / ultrafiltration process compared unfavourably to those for the standard process, as can be seen from Table 44.

Table 44. Effect of Steam Injection of Extract on UF Process Yields.

	Standard extraction/UF (Average of 3 runs)	Extraction / Steam injection / UF
% yield (solids)	28.7	20.8
% yield (protein)	52.9	37.6

The lower yield is due to the additional processing step and also to the heat treatment of the extract which causes appreciable losses of protein in the ultrafiltration permeate. It was concluded that heat treatment of the extract prior to ultrafiltration cannot be justified.

9.2.3 Discussion

It was shown that, in the case of the isoelectric process samples, T.I.A. values could be further reduced to levels similar to those of the commercial isolates, however levels in the UF process samples could not readily be reduced without considerable detrimental effects on process efficiency. It can be concluded that:

i. the four commercial isolates studied are probably all produced by the isoelectric process.

ii. UF process isolates are unacceptable from the point of view of T.I.A. It should be noted however that recent studies (Kennedy 1994) have suggested that trypsin inhibitor may have anti-carcinogenic properties. It follows therefore that, providing the levels of trypsin inhibitor consumed in the diet are not sufficiently high to cause major deficiencies in protein metabolism, consumption of soya protein containing residual T.I.A. may actually be beneficial. Furthermore, all the studies relating to the undesirable effects of T.I.A. have been carried out on animals. No studies in this field have been carried out using human subjects.

9.3 PHYTATE

9.3.1. Literature Review

Phytic acid, defined as myo-inositol hexaphosphate, has long been known to occur in sources of vegetable protein. It is known to form insoluble complexes with a number of essential mineral elements, among them calcium, magnesium, iron and zinc thus reducing the bioavailability of those minerals. The occurrence of phytate in soya protein products has been reviewed (de Boland et al 1975, Jaffee 1981, Erdman & Forbes 1981). The isoelectric process was found to result in retention of phytate in the resulting isolate (Johnson & Kikuchi 1989). However, Hartman (1979) managed to reduce phytate levels in isolates by ultrafiltration at 65°C. Okubo et al (1975) used a two stage process incorporating dissociation of protein/phytate complexes at low pH followed by ultrafiltration to remove phytate from isolates. Omosaiye & Cheryan (1979) used ultrafiltration at neutral pH followed by dilution and re-ultrafiltration to remove more than 90% of the phytate content of aqueous soya extracts. Brooks and Morr (1982) used an ion exchange method to remove 96 - 97% of the phytate content of soya protein isolate.

Phytate content of cereals is normally determined by the method of Wheeler & Ferrell (1971). The method involves extraction of the phytate with trichloracetic acid, precipitation of the phytate as ferric phytate and analysis of the precipitate for iron, which is then correlated to the phytate content in terms of the iron : phosphorous molecular ratio. However, Thompson & Erdman (1982) adapted this method by analyzing for phosphorous in the ferric phytate and modifying extraction procedures to improve repeatability of results. Their method was used specifically for soya products and was therefore adopted for the purposes of this study.

9.3.2 Method

The method of Thompson & Erdman (1982) was used .

REAGENTS

i. Extraction solution. Dissolve 30g trichloroacetic acid (TCA) and 100g sodium sulphate in 1ℓ distilled water.

ii. Phytate precipitator solution. Dissolve 2g ferric chloride and 16.3 ml concentrated hydrochloric acid in 1ℓ distilled water.

iii. Wash solution. Dissolve 20 ml concentrated hydrochloric acid and 25g sodium sulphate in 1ℓ distilled water.

iv. Ammonium vanadate solution. Dissolve 2.5g AR ammonium vanadate in 500ml boiling distilled water. Partially cool, add 20 ml concentrated AR nitric acid and when cool make up to 1ℓ .

v. Nitric acid - 1 part conc nitric acid + 2 parts distilled water.

vi. Ammonium molybdate solution. Dissolve 50g AR ammonium molybdate in 500ml distilled water at 70°C and make up to 1ℓ when cold.

vii. Standard phosphorous solution. Dissolve 4.393g AR potassium dihydrogen phosphate (previously dried at 105°C for 2 hours) in 1ℓ of distilled water. Dilute 50 ml of the solution to 1ℓ to obtain a diluted standard solution (1 ml = 0.05 mg P)

PROCEDURE : PHYTATE EXTRACTION.

i. Place an accurately weighed sample (approximately 2g) of material in a 250 ml flask, add 100ml extraction solution, stopper the flask and shake for 2 hours using a mechanical shaker.

ii. Filter off the extract and pipette 10ml of filtrate into a 50 ml centrifuge tube.

iii. Add 10 ml distilled water and 12 ml of the phytate precipitation solution. Heat in a boiling water bath for 75 minutes then cool to room temperature.

iv. Centrifuge for 15 min at 2000 rpm. Decant off the supernatant and wash the precipitate three times with 25 ml portions of the wash solution, centrifuging for 10 minutes at 2000 rpm each time and discarding the supernatant.

v. Add 10 ml conc nitric acid to the washed precipitate of ferric phytate and transfer the mixture to a 250 ml beaker, using a small amount of distilled water to rinse the centrifuge tube.

vi. Add four drops of concentrated sulphuric acid to the beaker and evaporate to dryness on a hot plate.

vii. Add 4 - 5 ml of 30% hydrogen peroxide and continue heating until bubbling ceases.

viii Dissolve residue in 15ml 3N hydrochloric acid and heat gently for 15 minutes. Cool and make up to 100 ml.

DETERMINATION OF PHOSPHOROUS CONTENT

i. Take 20 ml aliquot of phytate solution, add 5 ml concentrated nitric acid and evaporate to dryness. Add further 5 ml concentrated nitric acid and again evaporate to dryness.

ii. Dissolve residue in 10 ml nitric acid and transfer to a 100ml volumetric flask.

iii. Add 10 ml ammonium vanadate soln followed by 10 ml ammonium molybdate soln . Shake well and make up to 100 ml.

iv. Determine the optical density of the solution at 430 nm.

v. Prepare a series of standard phosphorous solutions by transferring aliquots of diluted standard solutions to 100 ml volumetric flasks, adding 10 ml nitric acid, 10 ml ammonium and 10 ml mylbdate soln ammonium vanadate soln, shaking and making up to 100 mls.

vi. Determine the optical densities of the standard solutions and use them to plot a graph from which the concentration of phosphorous in the test solution may be determined.

$$\%\ \text{phytate} = \text{mg P/20 ml aliquot (determined from graph)} \times \frac{100}{20} \times \frac{100}{10} \times \frac{1}{0.282} \times \frac{1}{\text{sample wt. (mg)}} \times 100\%$$

The conversion factor of 0.282 for the phosphorous content of phytic acid is that used by de Boland et al (1975).

Determinations were carried out in duplicate on all samples.

A similar range of samples to that used for trypsin inhibitor activity was used, namely defatted soya flakes, experimental samples from the three processes, commercial products and the standard and best process extracts. Since the spraydrying process is unlikely to influence phytate levels in isolates, it was not considered necessary to carry out phytate determinations of the isoelectric and UF products prior to spraydrying.

A preliminary recovery study using a sample containing a known quantity of phytate was carried out. A recovery of 99.0% was achieved, confirming the acceptability of the proposed method.

9.3.3 Results

These are shown in Table 45.

Table 45. Phytate Content of Experimental and Commercial Soya Isolate Samples.

SAMPLE	% Phytic Acid	% Phytic Acid/ 100g protein
Defatted soya flakes	1.65 ± 0.04	3.32 ± 0.08
Isoelectric process (dry product)	2.08 ± 0.19*	2.45 ± 0.22*
Ultrafiltration process (dry product)	1.59 ± 0.08*	1.82 ± 0.09*
Swollen gel process (solubilised product)	2.46 ± 0.32*	3.03 ± 0.39*
Commercial soya isolates :		
PP 500E	1.63 ± 0.22	1.87 ± 0.25
Supro 670	1.00 ± 0.02	1.08 ± 0.02
Supro 710	1.29 ± 0.02	1.39 ± 0.24
Ardex F	1.45 ± 0.08	1.60 ± 0.09
Standard extract (1 stage extraction)	1.59 ± 0.09	2.63 ± 0.15
Best process extract (2 stage extraction)	1.60 ± 0.04	2.58 ± 0.06

* Mean of 6 determinations (duplicate determinations for each of 3 standard samples from each process).

All other results are mean of 2 determinations.

9.3.4 Discussion.

Noticeable differences exist between phytate contents of the various samples but it is clear that none of the processes used substantially reduces phytate levels, although it can be seen from the figures for phytate content per 100g protein that some reduction takes place as the protein is concentrated.

The ultrafiltration process gives lower values than both the isoelectric and swollen gel processes and the commercial samples are all lower in phytate content than the experimental samples. In broad terms, phytate levels are in line with those observed elsewhere (de Boland et al 1975, Brooks & Morr 1982). It appears that differences in pH and temperature / time combinations between the three processes cause varying degrees of protein / phytate complexing thus causing the differences in phytate values.

The ultrafiltration process has been used for removal of phytate from soya protein extracts (Hartman 1979, Okubo et al 1975, Omosaiye & Cheryan 1979), however conditions used were substantially different from those adopted in this study.

Johnson & Kikuchi (1989) quote values of between 2.0 and 2.5% phytate for commercial soya isolates. The values obtained in this study are somewhat lower.

It can also be seen that choice of extraction process has no effect on product phytate content.

9.4 LYSINOALANINE

9.4.1 Literature Review

The presence of lysinoalanine [(N-(DL-2-amino-2-carboxyethyl)-L-lysine] in proteins subjected to severe alkali treatment was first reported in the mid 1960's (Patchornik & Sokolovsky 1964). Numerous further studies followed and the potential toxicity of this material was demonstrated by Newberne & Young (1966) who demonstrated the development of kidney defects in rats fed alkali treated soya proteins. Further studies relating to the effect of alkali treatment on protein quality (de Groot & Slump 1969) were carried out, as were further investigations into the potential toxicity of lysinoalanine (de Groot et al 1976). The occurrence of lysinoalanine in food products and its potential toxicity has been extensively reviewed (Struthers 1981, Friedman et al 1984, Maga 1984) and, while conclusive evidence of human toxicity has not been shown, sufficient concerns exists for the presence of lysinoalanine in foodstuffs to be generally considered undesirable.

In the specific case of soya protein isolate, the use of alkaline conditions for the initial extraction process indicates the potential for lysinoalanine formation. However, published studies on lysinoalanine formation by alkali treatment of soya protein (Hasegawa & Okamoto 1980, Hayashi & Kameda 1980) generally used more severe conditions in terms of time, temperature and alkali concentration than those used for routine solubilisation of soya protein. Of greater concern is the ultrafiltration process, where an alkaline solution of the protein is kept at fairly elevated temperatures for extended periods during the separation process. These conditions are more conducive to lysinoalanine formation, however no studies appear to have been published in this field.

Considerable effort has gone into development of methods for the qualitative and quantitative determinations of lysinoalanine. The most common methods involve ion- exchange chromatography of hydrolysed proteins followed by colorimetric determination with ninhydrin (Robson et al 1967, Rayond 1980). Methods using thin-layer chromatography (Sternberg et al 1975) and high-pressure liquid chromatography (Wood-Rethwill & Warthesen 1980) have also been proposed.

9.4.2. Experimental

It was initially intended to determine lysinoalanine contents of the soya isolate samples by means of ion-exchange chromatography of the hydrolysed proteins followed by colorimetric determination using ninhydrin. This would have enabled lysinoalanine determination to have been carried out at the same time as amino acid analysis (see chapter 10). However the Department of Animal and Poultry Science at the University of Natal in Pietermaritzburg, who were carrying out the amino acid analysis of the samples, were unable to perform lysinoalanine determinations, due to their heavy workload and consequent unwillingness to undertake modifications to their standard amino acid determination procedure.

An outside consulting laboratory was therefore commissioned to perform lysinoalanine analysis using the high pressure liquid chromatography method of Wood-Rethwill and Warthesen (1980). The method involves preparation of the dansyl derivatives of the hydrolysed proteins, isolation and detection of the dansyl derivative of lysinoalanine by high pressure liquid chromatography and quantification of lysinoalanine by comparison with a reference standard of lysinoalanine derivatised in similar fashion to the test samples. This method is

however less well established than that originally proposed and considerable difficulty was experienced in obtaining satisfactory results. Further difficulty was experienced due to lack of existing published data for typical lysinoalanine contents of soya protein isolates with a figure of 0 - 370 μg/g protein being the only one available (Maga 1984). Results obtained from the experimental samples differed very widely, showed poor repeatability, and in most cases were far higher than the existing published figures.

It was eventually reluctantly concluded that the results obtained were insufficiently reliable to guarantee scientific accuracy, consequently the results obtained for this part of the study are not included here.

10. AMINO ACID ANALYSIS OF SOYA PROTEIN ISOLATES

10.1 LITERATURE REVIEW.

Numerous studies have been carried out incorporating the determination of the amino acid control of soya protein isolate. Bau et al (1978) determined the amino acid composition of various fractions of soya protein isolates produced by various isoelectric precipitation methods. Kapoor & Gupta (1977) used physical separation of soya bean components and solubilisation of the protein fraction of those components to obtain samples for amino acid analysis. Morr (1981) obtained extensive amino acid data for soya proteins as part of an investigation into nitrogen conversion factors. Studies have also been carried out on the effect of processing on soya protein amino acid composition (Marshall et al 1982, Friedman et al 1984). The amino acid composition of soya protein isolates produced specifically by ultrafiltration has been extensively studied (Lawhon et al 1981, Lawhon et al 1982, Lawhon & Lusas 1984). Results of all these studies give broadly similar patterns for amino acid composition of soya proteins with variations observed only when severe processing conditions, particularly high temperatures, are used.

Methodology for amino acid acid analysis invariably involves hydrolysis of the proteins followed by use of an automated amino acid analyzer working on an ion-exchange chromatographic principle.

10.2 EXPERIMENTAL

Amino acid analysis of soya protein isolate samples was carried out by the Department of Animal and Poultry Science of the University of Natal in Pietermaritzburg.

10.2.1 Hydrolysis:

Samples (25 mg) were measured into a rimless pyrex test tube (12 x 150 mm) and 3 ml 6M hydrochloric acid was added. The mixture was frozen in an acetone/dry ice mixture, evacuated to less than 0.1 mm Hg, thawed under vacuum, refrozen and the tube was sealed in a flame, while the pressure was less than 0.1 mm Hg. Hydrolysis of the sample was effected at 110°C for 24 hours and, after cooling the tube was opened and norleucine internal standard solution (0.4 ml) was added and thoroughly mixed in. The hydrolysate was filtered through glass fibre filter paper and evaporated twice to the point of dryness at 40-45°C under reduced pressure in a rotary evaporator, before being made up to 5 ml with pH 2.2 buffer.

10.2.2 Amino Acid Analysis

Samples of the hydrolysate (0.25 ml) were analyzed in a Beckman 119 amino acid analyzer. The analyzer operates on the principle of ion-exchange chromatography on sulphurated polystyrene resin with detection of the amino acids by means of the ninhydrin reaction. Colorimetric detection is carried out by monitoring of the optical density at 570 nm. Output from the colorimeter is recorded graphically, the peaks on the recorder each corresponding to a particular amino acid which is identified from its elution time. Peak areas are measured by means of an integrator

and converted to actual amino acid concentrations by reference to a standard run constructed using a sample of known amino acid composition. A correction factor for recovery of the internal standard (in this case norleucine) is applied to the resulting data.

Analysis was carried out on the following samples:

Isoelectric process soya isolate

Ultrafiltration process soya isolate

Swollen gel process soya isolate (liquid form)

PP500E commercial soya isolate

Wet residue from initial protein solubilisation process.

Analysis of the wet residue from the initial protein solubilisation process was carried out in view of the potential value of this by-product material as a protein source.

10.3 RESULTS

These are shown in Table 46.

All results are expressed as g amino acid / 100 g protein.

Table 46. Amino Acid Analysis of Soya Protein Isolates.

AMINO ACID	Isoelectric Process Sample	Ultra-filtration Process Sample	Swollen Gel Process Sample	PP500E	Residue from Solubil-isation
Aspartic acid	11.578	11.308	11.368	10.897	10.595
Threonine	3.143	3.291	3.467	3.416	4.437
Serine	4.120	4.172	4.819	4.420	5.004
Glutamic acid	20.713	19.777	20.534	19.514	16.685
Proline	5.944	6.163	5.335	5.240	5.264
Glycine	3.806	3.784	3.935	4.140	4.572
Alanine	4.912	5.335	4.454	4.518	5.939
Valine	4.895	5.142	5.048	5.411	5.581
Methionine	1.209	1.302	0.996	1.332	1.103
Isoleucine	4.714	4.776	4.726	5.133	4.728
Leucine	7.244	7.242	7.697	8.033	8.391
Tyrosine	3.622	3.603	2.944	3.581	3.762
Phenylalanine	5.056	4.924	5.328	5.269	4.865
Histidine	2.404	2.413	2.465	2.449	2.800
Lysine	5.906	6.019	6.064	6.125	7.434
Ammonia	3.371	3.360	3.368	3.552	3.007
Arginine	7.363	7.388	7.069	6.971	5.833
% Protein of sample	77.9	84.4	7.8	78.5	4.6

10.4 DISCUSSION

The following features are noticeable in the above results:

1. Amino acid compositions of the isoelectric and ultrafiltration process samples are very similar, with appreciable differences only noticeable for glutamic acid and alanine.
2. The swollen gel sample differs in several respects from the other two experimental samples with the low level of methionine particularly noticeable. Higher serine and lower proline, alanine and tyrosine values are also apparent.
3. The commercial PP500E isolate has lower levels of aspartic acid, glutamic acid, and proline than any of the experimental samples but appreciably higher levels of glycine, valine, isoleucine and leucine.
4. The solubilisation residue contains higher levels of serine, threonine, glycine, alanine, leucine, histidine and lysine but lower levels of aspartic acid, glutamic acid, ammonia and arginine than any of the isolate samples. The high level of lysine indicates that the residue may be of considerable commercial value, as its protein quality is good.

It is apparent that the various process stages have differing effects on the protein fractions of the soya and this is reflected in the varying amino acid compositions of the different products. The differences are generally speaking more apparent in this case than was observed for the results obtained by SDS-PAGE (See 11.4).

In view of the particular importance of the essential amino acids lysine and methionine in determining the overall quality of the protein, it is of value to compare the results obtained for these particular amino acids with those obtained in existing studies. (Table 47)

Table 47. Comparison of Amino Acid Analysis Figures with Analysis Figures from Existing Studies.

	Lysine (g/100g Protein)	Methionine (g/100g Protein)
Isoelectric process samples:		
This study	5.91	1.21
Bau et al (1978)	5.46	1.36
Marshall et al (1982)	5.66	1.41
Morr (1981)	5.82	1.92
Ultrafiltration process samples:		
This study	6.02	1.30
Lawhon et al (1981)	6.1	1.3
Swollen gel process (this study)	6.06	1.00
Commercial soya isolates:		
This study (PP500E)	6.13	1.33
Marshall (1982)(unspecified)	6.11 6.12 6.30 6.16	1.37 1.32 1.34 1.28
Morr (1981) (unspecified)	5.27	1.65

Agreement with published studies for isoelectric process samples is reasonably good, except for the value for methionine obtained by Morr (1981) which appears to be extremely high. Agreement with published studies for the ultrafiltration process samples is also good.

It can be concluded that results obtained for amino acid analysis are generally in agreement with existing data.

11. POLYACRYLAMIDE GEL ELECTROPHORESIS (PAGE) OF SOYA PROTEIN ISOLATES.

11.1 LITERATURE REVIEW

The technique of polyacrylamide gel electrophoresis (PAGE) has been extensively used to characterise the different fractions of soya and other oilseed proteins. Kapoor & Gupta (1977) compared electrophoretic patterns and amino acid composition of proteins from different soya bean varieties while McWatters & Cherry (1977) attempted to correlate data from gel electrophoresis with quantitative measurement of functional properties of soya bean and other flours. A comparison between electrophoretic patterns of commercial (presumably isoelectric process) and experimental ultrafiltration process isolates has been made (Lawhon et al 1979). The electrophoretic patterns of soya proteins produced by varying isoelectric precipitation/separation sequences have been studied (Honig & Wolf 1987). Recent studies (Arrese et al 1991, Sorgentini et al 1991) have used the method of Laemmli (1970) to compare different commercial soya isolates and study the effect of denaturation of the protein on electrophoretic patterns.

11.2 EXPERIMENTAL PROCEDURE

Gel electrophoresis of samples was carried out by staff of the Department of Physiology of the University of Natal Medical School.

The procedure was carried out on a Hoeffer SE 250 slab gel electrophoresis unit using an adaptation of the discontinuous sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS - PAGE) method of Laemmli (1970).

The following samples were used:

Isoelectric process soya isolate : Samples from 3 standard process batches (designated 1,2,3).

Ultrafiltration process soya isolate: Samples from 3 standard process batches (designated 1,2,3)

Swollen gel process soya isolate: Samples from 3 standard process batches (designated 1,2,3).

Commercial soya isolates : PP 500E, Supro 670, Supro 710, Ardex F

Solubilised extracts : Standard (1 stage), Best Process (2 stage)

Isoelectric process extract (prior to spraydrying)

Ultrafiltration process extract (prior to spraydrying)

The solid samples were dispersed in water using a Silverson mixer. The resulting dispersions and the other samples (already in liquid form) were clarified by high speed centrifugation, first at 10000G then at 14000G. Samples were then assayed for protein content using the method of Bradford (1976) before carrying out the SDS-PAGE procedure.

11.2.1 Bradford Method for Protein Determination

REAGENTS:

1. Bradford Reagent. Serva blue G (25mg) is completely dissolved in 25 ml of 85% (w/v) phosphoric acid. Absolute ethanol (12 ml) is added to this and completely mixed in, and the solution is diluted to 250 ml with distilled water.
2. Protein Standard. Ovalbumin (1 mg/ml).

PROCEDURE:

Bradford reagent (5 ml) is added to the protein solution (0,1 ml). The mixture is vortexed and allowed to stand for 2 min before its absorbance at 595 nm is measured. A standard curve is constructed from quintuplicate assays at five levels between 20-100 μg protein, using 0,15M NaCℓ as a blank.

11.2.2 Method for SDS-PAGE of Soya Protein [Adapted From Method of Laemmli (1970)]

REAGENTS:

Solution A : Monomer solution (30% (w/v) acrylamide, 2,7% (w/v) bis-acrylamide). Acrylamide (58,4 g) and bis-acrylamide (1,6 g) were dissolved in 150 ml distilled water and made up to 200 ml with distilled water.

Solution B: Running Gel Buffer (1,5 M Tris-HCℓ, pH 8,8). Tris (36,6 g) was dissolved in 150 ml distilled water, titrated to pH 8,8 with HCℓ and was made up to 200 ml.

Solution C: 4 x Stacking Gel Buffer (50 mM Tris-HCℓ, pH 6,8). Tris (12 g) was dissolved in 150 ml distilled water titrated to pH 6,8 and was then made to 200 ml.

Solution D: 10% (w/v) SDS. SDS (10 g) was dissolved in 100 ml distilled water.

Solution E: Initiator (10% (w/v) ammonium persulphate). Ammonium persulphate (0,5 g) was dissolved in 100 ml distilled water immediately before use.

Solution F: Tank buffer (200 mM Tris-HCℓ, 0,192 M glycine, 0,1 % (w/v) SDS, pH 8,3). Tris (12 g) and glycine (57,5 g) were dissolved in distilled water and made up to 4ℓ. Prior to use in SDS denaturing PAGE, 2,5 ml of SDS stock solution (Solution E) was added to 250 ml of tank buffer.

Solution G: Treatment Buffer (0,125 M Tris-HCℓ, 4% (w/v) SDS, 20% (v/v) glycerol and 10% (v/v) 2-mercaptoethanol). Solution C (2,5 ml), solution D (4 ml), glycerol (2 ml) and 2-mercaptoethanol (1 ml) were mixed and made up to 10 ml with distilled water. The solution was then divided into 1 ml portions and frozen until needed.

Stain Stock Solution (1 % (w/v) Coomassie blue R-250):

Coomassie blue R-250 (1 g) was dissolved in 100 ml distilled water with the aid of magnetic stirring (1 hr) at room temperature, filtered through No. 1 filter paper and stored until needed.

Staining Solution (0,125% (w/v) Coomassie blue R-250, 50% (v/v) methanol, 10% (v/v) acetic acid):

Stock stain solution (62,5 ml) was mixed with methanol (250 ml) and acetic acid (50 ml) and made up to 500 ml with distilled water.

Destaining Solution 1 (50% (v/v) methanol, 10% (v/v) acetic acid):

Methanol (500 ml) was mixed with acetic acid (100 ml) and the volume was made up to 1ℓ with distilled water.

Destaining Solution 2 (17% acetic acid, 5% methanol):

Acetic acid (70 ml) was mixed with methanol (50 ml) and the volume was made up to 1ℓ with distilled water.

Solutions A, B and C were filtered through No. 1 filter paper before use. Preparation of 9% separating gel: 15 ml of solution A, 3,375 ml of solution B, 6,5 ml of distilled water, 0.15 ml of solution D, 75 $\mu\ell$ of solution E and 7.5 $\mu\ell$ of tetramethyl - ethylenediamine (TEMED) were mixed together in the above order. The gel was poured immediately after mixing.

Preparation of 4% stacking gel : 0.94 ml of solution, 1.75 ml of solution C, 4.2 ml of distilled water, 70 $\mu\ell$ of solution D, 35 $\mu\ell$ of solution E and 10 $\mu\ell$ of TEMED were mixed together in the above order.

PROCEDURE:

The Hoefer SE 250 apparatus was assembled according to the manufacturer's instructions. One notched aluminium plate and glass plate were washed with ethanol for each side of the apparatus. The glass and aluminium plate separated by two plastic spacers (1.5 mm) at the edges were clamped to the apparatus. The bottom space was filled with molten agar (1%, v/v) which was allowed to solidify. Once solid, running gel solution was run into the spaces between the glass and aluminium plates to a depth of 3 cm from the top of the glass plate. The running gel was overlaid with water to allow for even polymerisation. Once the running gel had set (approximately 45 min) water was poured out and stacking gel solution was poured over it up to the notch on the aluminium plate. Into the stacking gel solution a 10 well comb was inserted to allow for the formation of sample application wells. Once the stacking gel had set the comb was removed and the newly formed wells were rinsed with distilled water.

Tank buffer (with SDS for SDS denaturing PAGE) was poured into the lower and upper electrode compartments of the PAGE equipment. Sample (20 - 30 $\mu\ell$) was applied to the wells with a Hamilton Microsyringe. The Hoefer apparatus was then connected to a power pack and run at 18 mA per gel until the bromophenol blue tracker dye was approximately 0,5 cm from the bottom of the running gel. The apparatus was then disconnected from the power supply, plates

unclamped and levered apart using the plastic spacers. The gels were removed from the plates and placed in stain solution for four hours. After four hours the stain was removed and the gel was placed into destain 1 solution overnight, followed by further destaining in destain 2 solution until destaining was complete.

11.3 RESULTS

Protein concentrations used are shown in Table 48.

Table 48. Protein Concentrations for SDS-PAGE Process.

SAMPLE		Protein Concentration	
Isoelectric process samples:		20	μg/lane
Ultrafiltration process samples:		25	μg/lane
Swollen gel process samples:		16	μg/lane (Batch 1)
		18	μg/lane (Batch 2, Batch 3)
Commercial soya isolates:	PP500E	15	μg/lane
	Supro 670	14	μg/lane
	Supro 710	12	μg/lane
	Ardex F	20	μg/lane
Solubilised extracts:	Standard	10	μg/lane
	Best Process	11	μg/lane
Isoelectric process extract		8	μg/lane
Ultrafiltration process extract		9	μg/lane

The electrophoresis patterns obtained are shown in figs. 8 - 12. Fig. 8 gives the pattern observed for the standard molecular weight markers used and table 49 gives details of the markers.

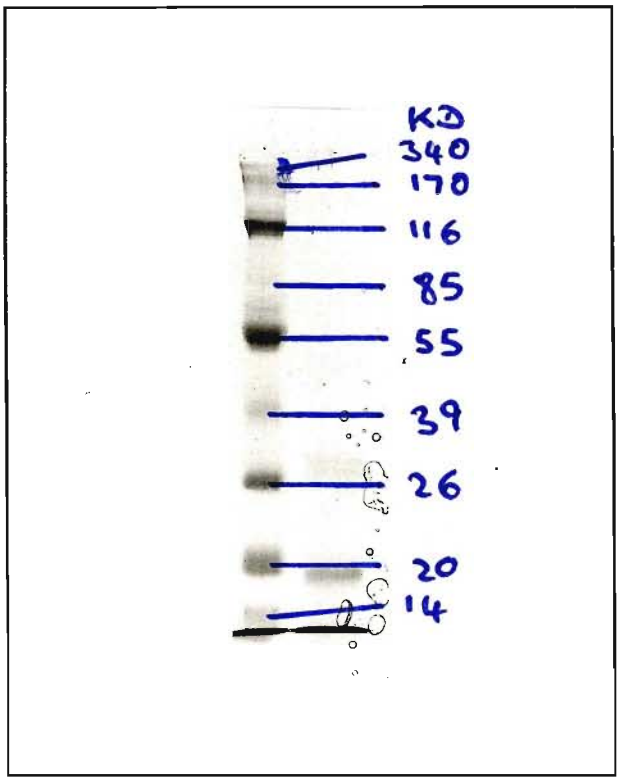

Fig. 8. Molecular weight markers.

The calibration proteins used are listed in Table 49.

Table 49. Calibration Proteins for Molecular Weight Markers.

Calibration protein used	Molecular weight
α_2 macroglobulin (horse plasma)	340000 (non reduced) 170000 (reduced)
B - ß - Galactosidase (E - coli)	116353
Fructose - 6 - Phosphate Kinase (Rabbit muscle)	85204
Glutamate Dehydrogenase (Bovine liver)	55562
Aldolase (Rabbit muscle)	39212
Triose phosphate isomerase (rabbit muscle)	26626
Trypsin inhibitor (soybean)	20100
Lysozyme (hen egg white)	14307

Fig. 9 gives the patterns for isoelectric and ultrafiltration process samples and their respective extracts.

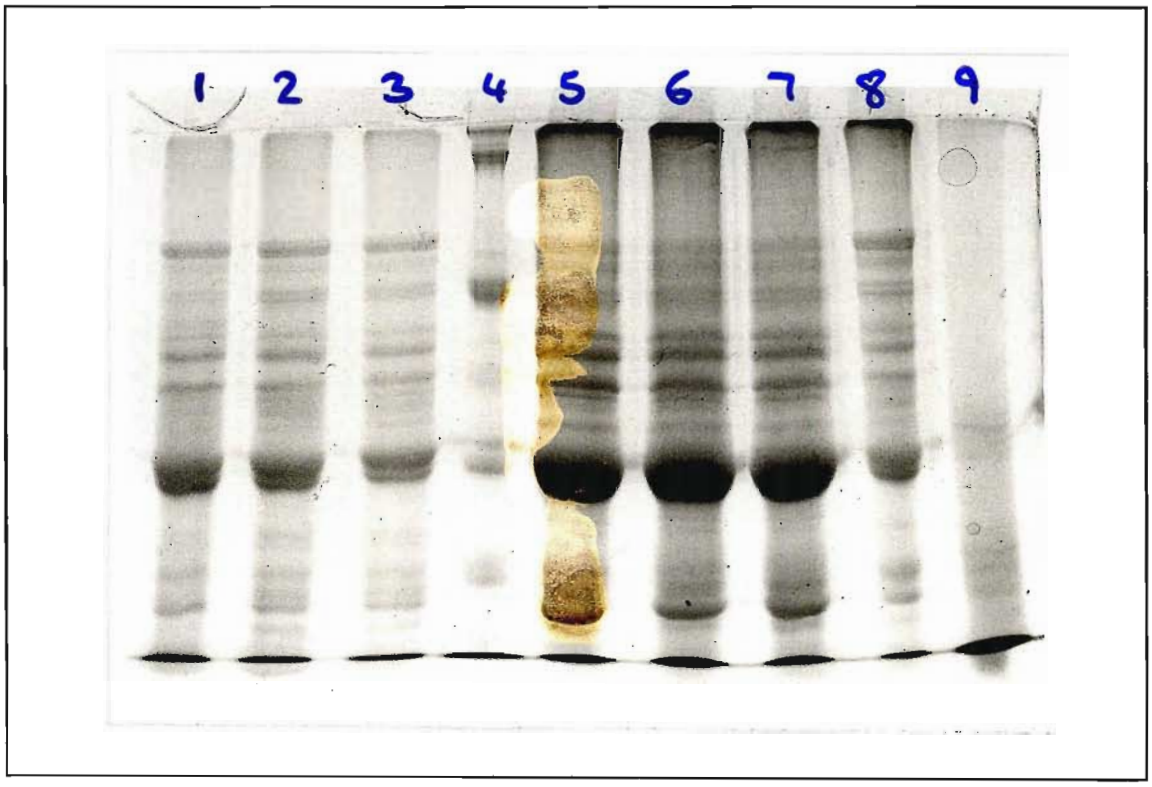

Fig. 9. SDS-PAGE Results for Isoelectric and UF Process Samples.

Fig. 9.	Lane	Sample.
	1	Isoelectric process batch 1
	2	Isoelectric process batch 2
	3	Isoelectric process batch 3
	4	Molecular weight marker
	5	Ultrafiltration process batch 1
	6.	Ultrafiltration process batch 2
	7.	Ultrafiltration process batch 3
	8.	Isoelectric process extract
	9	Ultrafiltration process extract.

Fig. 10 gives a comparison of the patterns for the isoelectric and ultrafiltration process samples with those of the standard and best process solubilised extracts.

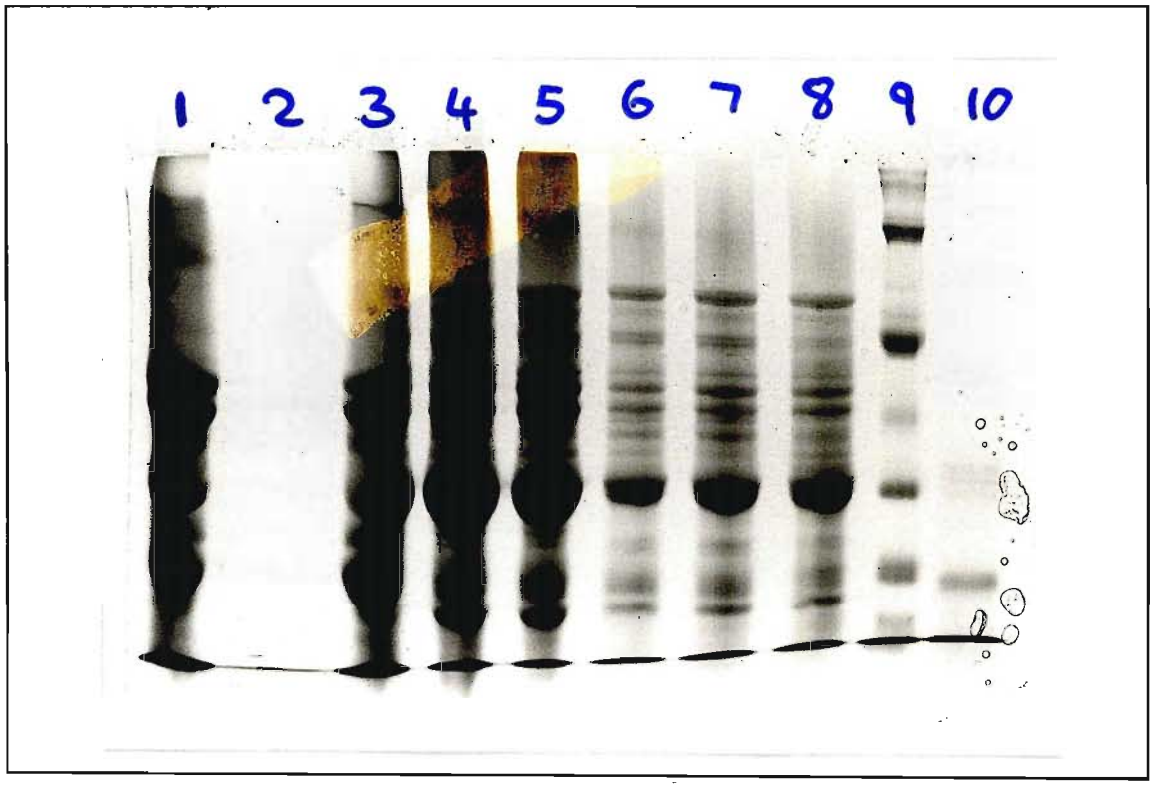

Fig. 10. SDS-PAGE Results for UF and Isoelectric Process Samples and Solubilised Extracts.

Fig.10	Lane	Sample.
	1	Best process extract
	2	No sample
	3	Ultrafiltration process batch 3
	4	Ultrafiltration process batch 2
	5	Ultrafiltration process batch 1
	6	Isoelectric process batch 3
	7	Isoelectric process batch 2
	8	Isoelectric process batch 1
	9	Molecular weight markers
	10	Standard process solubilised extract

Fig. 11. gives a comparison of the patterns for the commercial soya products with those for the swollen gel process samples and the isoelectric and ultrafiltration process extracts.

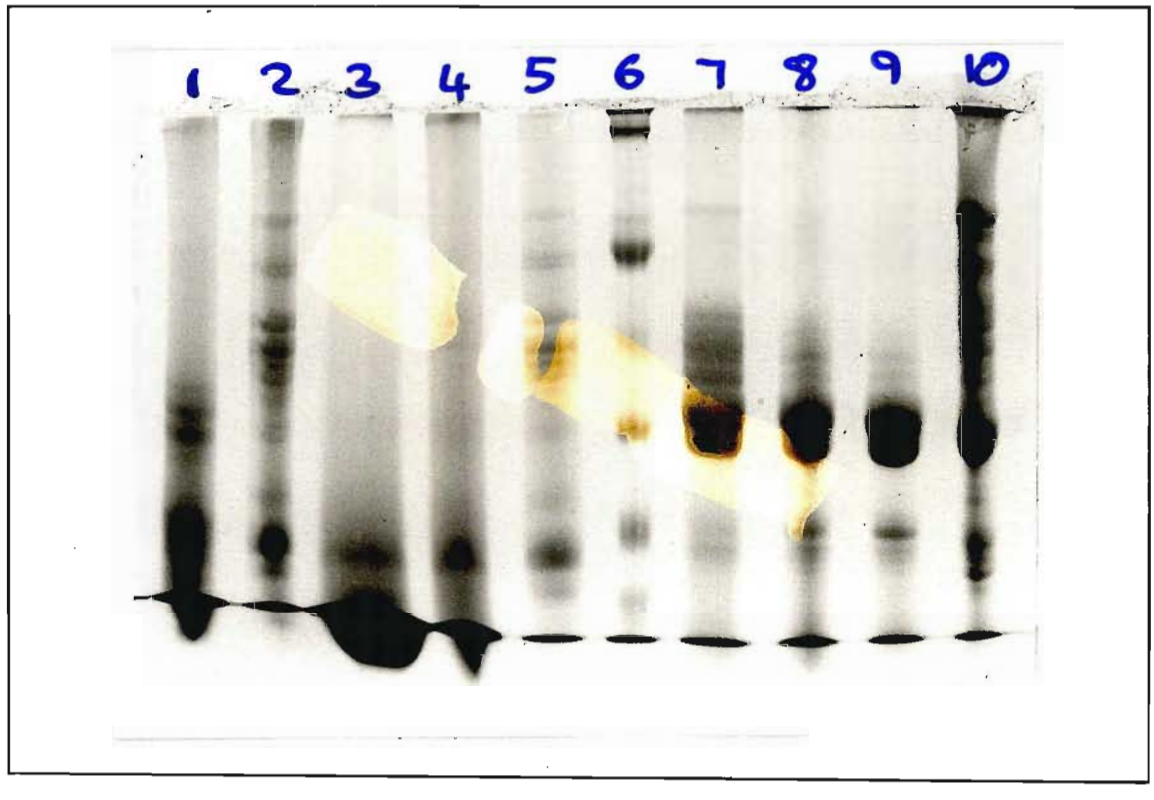

Fig. 11. SDS-PAGE Results for Commercial Isolates, Swollen Gel Process Samples and Isoelectric/UF Process Extracts.

Fig. 11. Lane	Sample
1	Ultrafiltration process extract
2	Ardex F
3	Supro 710
4	Supro 670
5	PP 500E
6	Molecular weight markers
7	Swollen gel process batch 3
8	Swollen gel process batch 2
9	Swollen gel process batch 1
10	Isoelectric process extract

Fig. 12 gives a further comparison of the patterns for the swollen gel process samples with those of the commercial products.

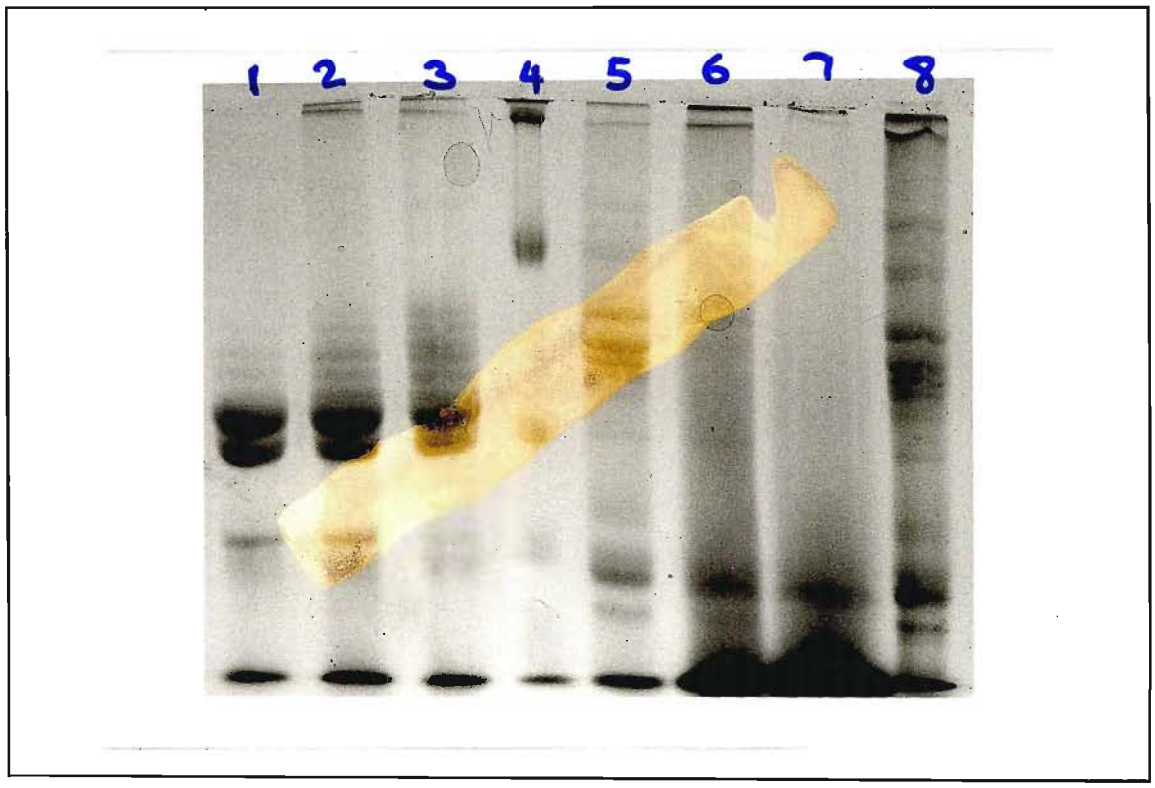

Fig. 12. SDS-PAGE Results for Swollen Gel Process Samples and Commercial Soya Isolates.

Fig. 12	Lane	Sample
	1	Swollen gel process batch 1
	2	Swollen gel process batch 2
	3	Swollen gel process batch 3
	4	Molecular weight markers
	5	PP500E
	6	Supro 670
	7	Supro 710
	8	Ardex F

11.4 DISCUSSION

The principal feature of note in the electrophoresis patterns obtained is the presence of trypsin inhibitor in the samples. The 20 KD marker in the standard molecular weight samples actually consists of trypsin inhibitor hence its presence can readily be detected.

Fig. 9 clearly indicates that the levels of trypsin inhibitor present in the ultrafiltration process samples are higher than those in the isoelectric process samples, confirming the results obtained by analysis (see 9.1). Fig. 10 provides further confirmation of this, however results for the solubilised extracts, ultrafiltration process extract and isoelectric process extract are not clear. Figs. 11 & 12 provide confirmation of the low levels of trypsin inhibitor in the swollen gel samples (see 9.1). Patterns for the commercial soya isolates (Figs. 11 & 12) are not well defined but indicate the possible presence of low levels of trypsin inhibitor.

Other features of note in the electrophoretic patterns are:

i. the presence of high molecular weight fractions (340000 KD) in all samples. This corresponds to the 11S Globulin fraction, generally considered to be the largest single fraction present in soya protein (Smith & Circle 1977).

ii. A series of fractions common to all samples in the range 40-170 KD. These can be attributed to fractions such as lipoxygenase (100 - 110KD), haemagglutenin (90 - 105 KD) and ß - amylase (60 - 70 KD).

iii. The presence of numerous fractions with molecular weights less than 50 KD in all samples, including those from the ultrafiltration process. This indicates that at least some proteins with molecular weight less than 50000 are not removed by the 50000 MWCO ultrafiltration membrane. This may be due to complexing with other higher molecular weight fraction as discussed in 9.1.

12. COMPARATIVE ECONOMICS OF ISOELECTRIC AND ULTRAFILTRATION PROCESSES FOR PRODUCTION OF SOYA PROTEIN ISOLATE.

Of fundamental importance in the choice of process is a comparison between the economic feasibility of the two processes. Tentative costs for the isoelectric process have been reported (Mustakas & Sohns 1978) as has a comparison between the two processes in economic terms (Hensley & Lawhon 1979).

A comparison between the processes should incorporate

i. Comparative capital costs.

ii. Comparative operating costs.

iii. Disposal of by-products from each process.

iv. Comparative yields of the processes in relation to their costs.

12.1 PLANT REQUIREMENTS

These have been based on a plant designed to produce 3000 tons/year of finished isolate, using approximately 11000 tons/year of defatted soya flakes.

12.1.1 Extraction Process

This is common to both processes. A typical production layout is summarised in Fig. 13.

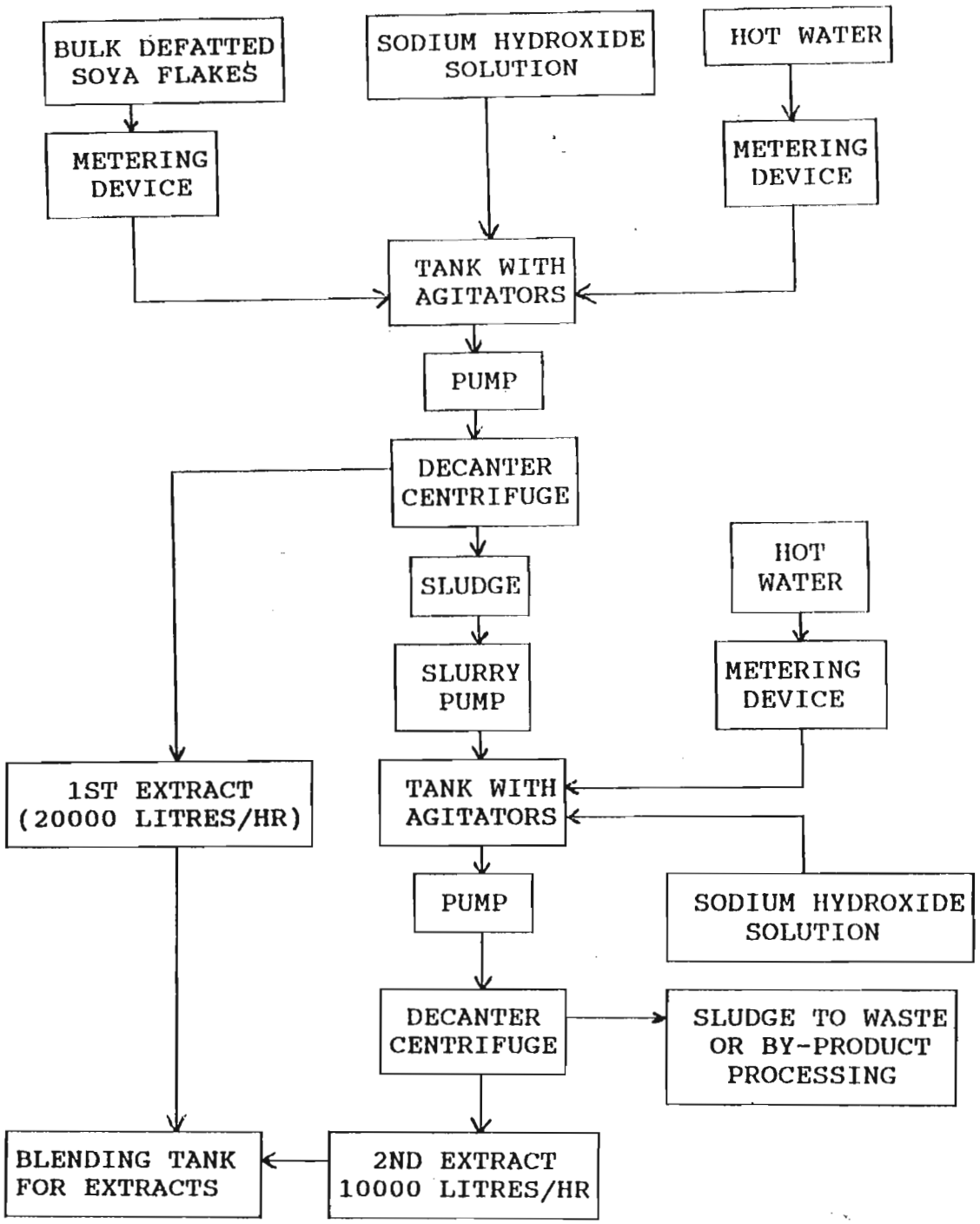

Fig.13. Two Stage Extraction Process.

Cost of the various items of equipment is estimated in Table 50.

Table 50. Plant Costs for Extraction Process.

ITEM	R	Potential Supplier
3 x 10000ℓ tanks with agitators (1st extraction)	70 000	NDE
Transfer pump (lst extraction)	15 000	NDE
Decanter centrifuge (1st extraction)	800 000	Alfa-Laval
Sludge pump (transfer to 2nd extraction)	33 000	NDE
2 x 11000ℓ tanks with agitators (2nd extraction)	50 000	NDE
Transfer pump (2nd extraction)	15 000	NDE
Decanter centrifuge (2nd extraction)	700 000	Alfa-Laval
Sludge pump (to waste or by-product processing)	33 000	NDE
Transfer pump (extract)	15 000	NDE
Pipework, valves etc., for liquid transfer	60 000	NDE
Instrumentation and control	40 000	NDE
TOTAL	R1 831 000	

12.1.2 Ultrafiltration Process

The production layout for the ultrafiltration process is summarised in Fig. 14.

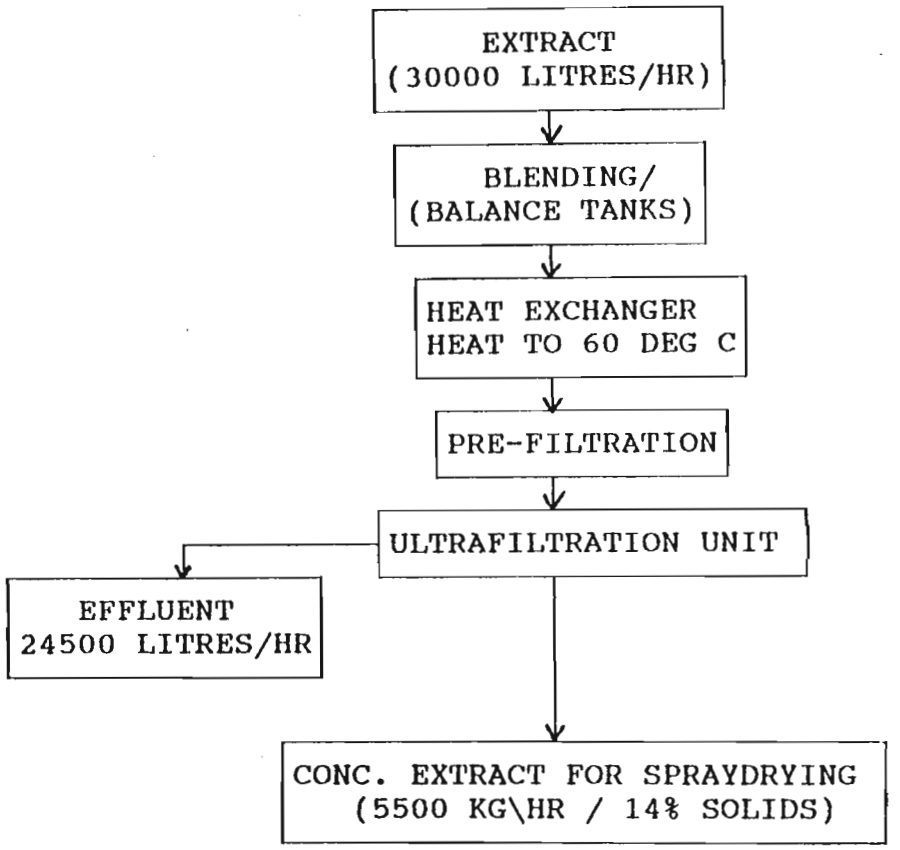

Fig. 14. Ultrafiltration Process.

Cost of the various items of equipment is estimated in Table 51.

Table 51. Plant Costs for UF Process.

ITEM	R	Potential Supplier
2 x 7500ℓ tanks (low speed agitators)	40 000	NDE
Transfer pump	15 000	NDE
Heat exchanger	18 000	Alfa Laval
Ultrafiltration unit (30000ℓ / hr)	16 000 000	Osmonics
Pipework, valves etc., for liquid transfer	15 000	NDE
Instrumentation and control	15 000	NDE
TOTAL	**R16 103 000**	

12.1.3 Isoelectric Process

The production layout for the isoelectric process is summarised in Fig. 15.

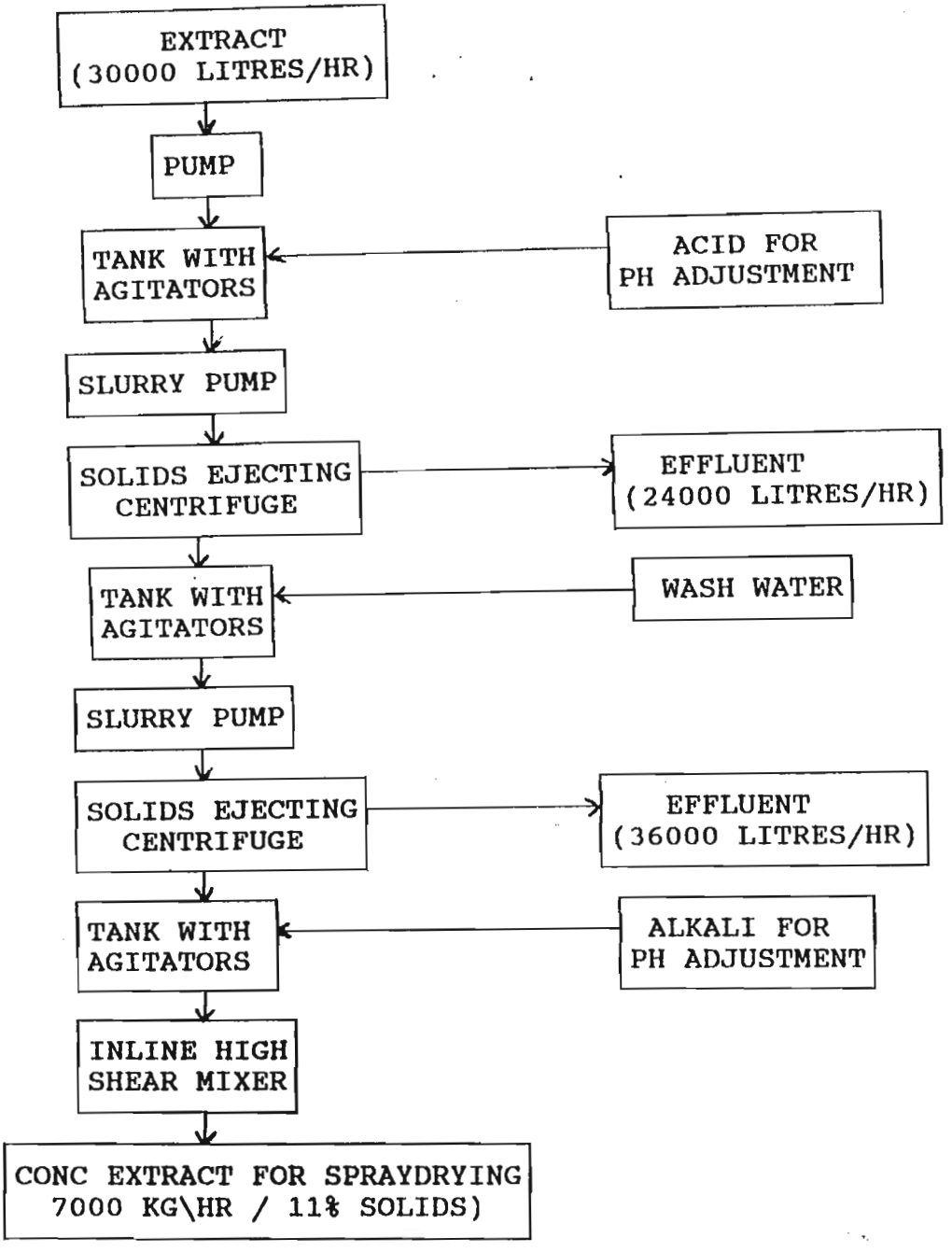

Fig. 15. Isoelectric Process

Cost of the various items of equipment is estimated in Table 52.

Table 52. Plant Costs for Isoelectric Process.

ITEM	R	Potential Supplier
2 x 15000ℓ tanks with agitators (pH adjustment)	56 000	NDE
Transfer pump	15 000	NDE
Solids ejecting centrifuge	500 000	Alfa Laval
Slurry pump	33 000	NDE
2 x 15000ℓ tanks with agitators (washing)	56 000	NDE
Transfer pump	19 000	NDE
Solids ejecting centrifuge	500 000	Alfa Laval
Slurry pump	33 000	NDE
2 x 4000ℓ tanks with agitators (pH adjustment)	26 000	NDE
High shear in line mixer	40 000	Silverson
Pipework, valves etc., for liquid transfer	60 000	NDE
Instrumentation and control	40 000	NDE
TOTAL	R1 378 000	

12.1.4 Spraydrying

This is common to both processes, however the isoelectric process produces a feed material lower in solids content than that from the ultrafiltration process. A typical production layout is summarised in Fig. 16.

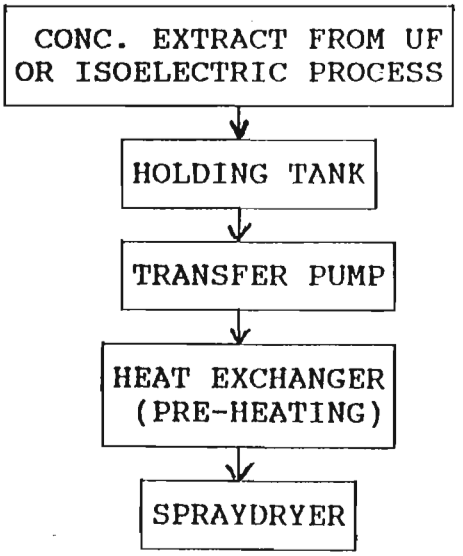

Fig. 16. Spraydrying Process.

Cost of the various items of equipment is estimated in Table 53.

Table 53. Plant Cost for Spraydrying Process.

ITEM	R	Potential Supplier
1 x 3000ℓ Holding tank	12 000	NDE
Transfer pump	15 000	NDE
Heat exchanger	18 000	Alfa Laval
Spraydryer	11 200 000*	Niro
Pipework, valves etc., for liquid transfer	15 000	NDE
Instrumentation and control	15 000	NDE
TOTAL	R11 275 000	

* It should be noted that it may be possible to use a smaller capacity spraydryer for the UF process, due to the higher solids content of the feed.

Total plant costs for the two processes can be summarised in Table 54.

Table 54. Summary of Plant Costs

Process	Extraction	Separation/ Concentration	Spraydrying	Total
Isoelectric process (R000)	1 831	1 378	11 275	14 484
Ultrafiltration process (R000)	1 831	16 103	11 275	29 209

12.1.5 Comments on Plant Costs.

A very sizeable discrepancy between the capital costs of the two processes is seen, due entirely to the high cost of the ultrafiltration plant. It should be noted however that costings for the commercial ultrafiltration plant are based purely on scaling up of the optimum process devised during the experimental trials (i.e. a three stage process incorporating pre-concentration, diafiltration and final concentration). If the process could be modified to remove the diafiltration step, cost of the plant would be halved. It has also been indicated that local fabrication of certain components of the ultrafiltration plant could reduce plant costs by a further 20 - 30%.

Implementation of these steps would reduce the cost of the ultrafiltration plant from R16 000 000 to approximately R6 000 000. Total plant cost for the ultrafiltration process would then reduce to R19 209 000, which is however still substantially higher than that of the isoelectric process.

12.1.6 Building Costs

Only a rough estimate of likely building costs can be made due to the lack of detailed information on plant dimensions and of the structure required to accommodate the spraydryer in particular. For costing purposes, a figure of R7 500 000 has been assumed for costs of both building and services (water, power, steam, compressed air) for both processes.

12.1.7 Total Capital Costs

These are summarised in Table 55.

Table 55. Total Capital Costs for Soya Isolate Plants.

	Isoelec. process	**UF process**	**Modified UF process**
Building cost (R000)	7 500	7 500	7 500
Plant cost (R000)	14 484	29 209	19 209
Total cost (R000)	**21 984**	36 709	26 709
Annual depreciation (buildings) (30 year life) (R000)	250	250	250
Annual depreciation (plant) (6.667 year life (R000)	2 172	4 381	2 881

12.2 OPERATING COSTS

These incorporate:

i. Cost of chemicals used

ii. Energy costs

iii. Maintenance costs

iv. Labour costs

v. Water costs

12.2.1 Chemicals

Estimated usage is:

Extraction process: 220 tons / yr 50% sodium hydroxide solution

@ R1 732/ton = R381 040 p.a.

Isoelectric process: 400 tons / yr concentrated hydrochloric acid

@ R955 / ton = R382 000 p.a.

20 tons / yr 50% sodium hydroxide solution

@ R1 732 / ton = R34 640.

Ultrafiltration process: Specialised membrane cleaning chemicals :

estimated cost R40 000 p.a.

Total chemical costs: Isoelectric process R798 000 p.a.

Ultrafiltration process R421 000 p.a.

12.2.2 Energy Costs

Electricity - estimated cost	R500 000 p.a. (isoelectric process)
	R750 000 p.a. (ultrafiltration process)
Steam - estimated cost	R200 000 p.a. (both processes)
Gas (for spraydryer) - estimated cost	R1 000 000 p.a. (ultrafiltration)
	R1 250 000 p.a. (isoelectric)

The difference is due to the greater drying capacity required for the isoelectric process product because of its lower feed solids content.

12.2.3 Maintenance Costs

These have been estimated according to:

Isoelectric process	- 3% of plant cost	=	R 435 000 p.a.
Ultrafiltration process	- Membrane replacement	=	R1 300 000 p.a.
	+ 1% of plant cost	=	R 300 000 p.a.
	TOTAL	=	R1 600 000

12.2.4 Labour Costs

Both proposed processes are capital rather than labour intensive, implying a small but highly skilled staff complement. Possible staffing levels could be:

1	x	Plant Manager
1	x	Production Manager
3	x	Shift Supervisors
15	x	Shift Operators
1	x	Engineering/Maintenance Supervisor
6	x	Engineering staff (Fitters/Electricians)
1	x	Quality Control Supervisor
3	x	Shift Quality Control staff
6	x	Cleaning/Service staff.

It is estimated that staffing requirements would be similar for both processes. Salary and staff benefit costs for the above are estimated to be R2 750 000 p.a.

12.2.5 Water Costs

Both processes use large quantities of water. These can be estimated as follows:

Initial extraction :	37 500 litres/hr
Ultrafiltration process:	No additional process water assuming no diafiltration required. Allow 3 000 litres/hr average for cleaning and sanitation.
Isoelectric process :	Wash water 37 000 litres/hr. Allow 2 000 litres/hr average for cleaning and sanitation.

Total water requirements are therefore:

Ultrafiltration process	40 500 litres/hr *
Isoelectric process	76 500 litres/hr

On the basis of proposed plant utilisation levels, water costs are estimated at:

Ultrafiltration process:	R300 000 p.a. *
Isoelectric process:	R600 000 p.a.

* It should be noted that, should a diafiltration stage be required as part of the ultrafiltration process, both water requirements and effluent volumes will increase substantially. Exact requirements cannot readily be specified without more detailed information on the extent of diafiltration required.

12.2.6 Total Operating Costs

Total annual operating costs for the two processes are summarised in Table 56

Table 56. Operating Costs for Soya Isolate Plants.

	Isoelectric Process (R000)	Ultrafiltration process (R000)
Chemicals	798	421
Electricity	500	750
Steam	200	200
Gas	1 250	1 000
Maintenance	435	1 600
Labour	2 750	2 750
Water	600	300
TOTAL	6 533	7 021

12.2.7 Comments on Operating Costs.

The ultrafiltration process appears to have slightly higher operating costs, due to the high cost of membrane replacement and higher electricity requirements. However the same considerations apply as in the case of the capital costs, namely the possibility of using a simpler ultrafiltration process without a diafiltration stage.

Removal of the diafiltration stage would halve both maintenance and electricity costs for the ultrafiltration process, reducing its annual operating costs to approximately R5 **800 000**. These costs are below those of the isoelectric process.

12.3 DISPOSAL OF BY-PRODUCTS

One of the greatest problems facing any manufacturer of soya isolate is the large volume of by-products generated, irrespective of the process used. Two particular by-products are involved.

1. Wet spent flakes from the extraction process.
2. Effluent from the isoelectric or ultrafiltration process.

12.3.1 Wet Spent Flakes

A plant designed to produce 3 000 tons/year of finished isolate will generate approximately 11 tons/hr of wet spent flakes. Both solids control and handling properties of the wet material will be dependent on the efficiency of the centrifugation process used to separate the flakes from the liquid extract. Typical values for the wet spent flakes obtained during experimental extractions were:

% solids	=	16.3
% protein	=	6.1
% protein (dry basis)	=	37.3

It can be seen that the wet flakes still have considerable value as a protein source, particularly in view of their high lysine content (see 10.3), however their high moisture content presents a disposal problem and a major potential microbiological and sanitary hazard. Potential methods of disposal are:

1. Incorporation in cattle feed 'as is' .
2. Incorporation in fortified foodstuffs, providing sanitary handling procedures are involved.
3. Dehydration for incorporation in cattle feed.

Methods 1 and 2 imply that the wet material can be immediately used in a subsequent process involving some form of dehydration, preferably on the same site as the isolate plant due to the very high susceptibility of the spent flakes to microbial spoilage.

Method 3 would involve a dedicated drying plant for the wet spent flakes. Drying load would be high due to the low solids content of the material .

12.3.2 Effluent

The following effluents would be expected from the two separation processes:

Isoelectric process : 60 000 litres/hr

Total solids	1.0%
of which dissolved solids	0.9%

Oxygen absorbance (OA) 700 mg/ℓ (by analysis)

pH 4.0 - 5.0

Ultrafiltration process: 24500 litres/hr

Total solids 2.5% (all solubilised)

Oxygen absorbance (OA) 1400 mg/ℓ (estimated)

pH 8.0 - 9.0

It can be seen that both the quantities of effluent and the treatment requirements (normal raw sewage has an OA of 60 - 70 mg/ℓ) are considerable. A number of options exist:

1. Direct discharge of effluent for municipal disposal. This could only be undertaken in those geographical areas where sufficient treatment capacity exists at the municipal sewage works. Informal discussions with several local authorities in the Durban area indicated that the high OA levels in the effluent and the large volumes of effluent involved would make direct discharge impossible in many municipalities. Durban municipality indicated that, in the event of the effluent being accepted for direct discharge, treatment costs would be:

   Isoelectric process : R1.57 / 1000 litres (R550 000 per year).

   Ultrafiltration process : R2.78 / 1000 litres (R420 000 per year).

2. In-house treatment prior to discharge. The process would involve the following stages:

   i. Settling tanks for separation of suspended solids (isoelectric process only)

   ii. pH adjustment, particularly for isoelectric process effluent.

   iii. Treatment in anaerobic ponds to reduce OA of effluent to a level where it can be discharged for municipal disposal.

3. Reverse osmosis treatment of effluent to yield:

   i. A concentrated effluent with a solids content of between 10 - 20%. Effluent solids composition on a dry solids basis is estimated to be approximately 20% protein for both processes, indicating a possible usage in cattle feed.

   ii. Water for re-cycling to the initial extraction process.

4. Re-cycling of effluent 'as is' for re-use in processing after suitable pH adjustment. The following factors would need to be considered.

   i. Cost of the recirculation system.

   ii. Microbiological considerations.

   iii. Effect of using re-cycled effluent on extraction and separation efficiencies.

   iv. Number of re-cyclings possible before final disposal of effluent.

   v. Eventual disposal of higher solids effluent after re-use.

On the basis of the above information, it is not possible to draw firm conclusions as to the most desirable method of effluent treatment or of the comparative merits of the isoelectric or ultrafiltration manufacturing processes in this regard. Should a full economic feasibility study for soya isolate manufacture be required, considerable further investigation of effluent treatment requirements will be necessary. For interim costing purposes, effluent treatment costs will be assumed to be those incurred for direct discharge (option 1 above).

12.4 RAW MATERIAL COSTS / COMPARATIVE YIELDS

Assuming a requirement for 3000 tons/year of soya isolate, raw material requirements will be dependent on the efficiency of extraction of the soya protein fraction for the two processes as determined in this study. The following yields are assumed:

	% solids yield
Initial 2 stage extraction.	58.0 (figure ex chapter 2.8)
Isoelectric process	52.9 (figure ex chapter 7)
Ultrafiltration process	55.8 (figure ex chapter 7)
Spraydrying (both processes)	97.0 (estimated figure)

Overall yields of soya protein isolate expressed as kg. soya protein isolate / 100 kg defatted soya flakes are therefore:

Isoelectric process	29.76 kg.
Ultrafiltration process	31.39 kg.

For 3000 tons/year soya protein isolate, raw material requirements per annum are therefore:

Isoelectric process	10 080 tons @ R1.49/kg*	=	R15 019 200 p.a.
Ultrafiltration process	9 557 tons @ R1.49/kg*	=	R14 239 930 p.a.

* Cost ex National Protein, Potgietersrus.

12.5 COST SUMMARY

The data obtained from 12.1 - 12.4 can be summarised to give an indication of the total production costs per annum for each process. In view of the possibility of modifying the ultrafiltration process to reduce both its capital and running costs, costs are given for both the standard and alternative ultrafiltration processes. Costs are summarised in Table 57.

Table 57. Total Cost of Soya Isolate Plants.

Item	Cost (R000 per annum)		
	Isoelec. Process	UF Process	Modified UF Process.
Building depreciation (12.1.7)	250	250	250
Plant depreciation (12.1.7)	2 172	4 381	2 881
Operating costs (12.2.6)	6 533	7 021	5 800
Effluent treatment (12.3.2)	550	420	420
Raw material costs (12.4)	15 019	14 240	14 240
Total	24 524	26 312	23 591
Cost/kg soya protein isolate (3×10^6 kg per annum)	R8.17	R8.77	R7.86

It should be noted that the above costs represent manufacturing costs only and exclude the following:

Packaging costs.

Distribution costs.

Sales/Marketing costs.

Any cost savings achieved by sale of by-products.

12.6 DISCUSSION

The ultrafiltration process as used for this study is less economical than the isoelectric process, due to its higher capital and operating costs, which are not sufficiently offset by the better yields of product obtained in the ultrafiltration process. However it can be seen that, by using the so-called modified ultrafiltration process, in which the diafiltration stage is removed or substantially reduced, manufacturing costs can be reduced to a level below those of the ultrafiltration process.

A further more detailed investigation into economic feasibility of soya isolate manufacture would require the following steps:

1. Detailed consideration of the economic implications of 1-stage versus 2-stage initial extraction processes and investigations into centrifugation requirements with a view to improving process yields.

2. A more detailed study of centrifugation requirements for the isoelectric process and investigations into improving centrifugation efficiency. The objective would be to raise the solids content of the final slurry to a level comparable to that obtained from the ultrafiltration process, thereby reducing spraydrying capacity requirements.

3. Investigations into the feasibility of the so-called modified ultrafiltration process in which the diafiltration stage is eliminated or substantially reduced.

4. Further consideration of effluent disposal and/or process water recycling.

5. Detailed investigations into the disposal of wet spent flakes from the initial extraction process.

The current cost of imported soya isolate ranges from R10 - R12/kg. It can be seen that, once packaging, distribution and sales/marketing costs have been added to the figures calculated in 12.5, along with a reasonable profit margin, total selling prices of locally produced soya isolates are unlikely to be lower than those of the imported products and may well be higher.

It would appear that substantial cost reductions could only be obtained by:

1. Obtaining a lower cost raw material
2. Increasing the plant capacity, thereby reducing overheads per kg. finished product.
3. Improving process yields for the extraction and separation stages.
4. Developing an outlet for by-products, in particular wet spent flakes which have appreciable commercial value as a protein source.

13. SUMMARY AND CONCLUSIONS

The findings of this study can be summarised in terms of the objectives set out in Chapter 1.

The conditions for solubilisation of the protein fraction of the defatted soya flakes were satisfactorily optimised - little adjustment was found to be necessary from the standard conditions used for reference purposes. It is likely than yields at this stage can be further improved if the crude separation methods used for pilot scale processing can be replaced by centrifugal separation as used for the small scale process optimisation - this is clearly seen from the lower yields achieved for bulk extraction relative to the small scale extraction under optimised conditions.

The optimum conditions for isoelectric precipitation of the protein were investigated and found to be comparable to those found in existing published studies. Choice of acid had little effect on yield. Satisfactory quantities of soya protein isolate were produced for further evaluation but process efficiency and yield were affected by the crude separation techniques used and by the practical difficulties experienced during spraydrying.

The ultrafiltration process for soya isolate production was found to be highly successful and the performance of the pilot unit was extremely good. Once the process had been optimised, the finished product compared favourably to its isoelectric process counterpart in terms of both protein content and process yield (although spraydrying yields were again erratic). It is believed that the

ultrafiltration process could be further optimised by reducing the extent of the diafiltration stage - this would substantially increase process efficiency with only a limited effect on the protein content of the finished product.

In contrast, the swollen gel process gave disappointing performance. The principles reported in previous studies were confirmed but the practical difficulties and poor yields obtained during the preliminary studies were such that pilot scale production of isolate by this process was not undertaken, although small- scale samples were produced for analysis purposes. It is very unlikely that this process could ever be commercially viable, even if the cost of the gel was substantially reduced.

Functional properties of the isoelectric process and ultrafiltration process samples were compared and a further comparison was made with a selection of commercial soya isolates. Functional properties of the two experimental products were found to be fairly similar with the commercial isolates showing varying degrees of functionality in the various categories studied. The relevance of some of the methodology used in relation to the actual process conditions under which soya protein isolates are used is questionable, and a full evaluation of the experimental isolates could only be undertaken if larger quantities of product were available for evaluation in typical formulations.

The comparative levels of trypsin inhibitor activity, phytate and lysinoalanine in the experimental samples were determined and compared with those of commercial soya isolates. Substantial differences in trypsin inhibitor activity levels were

observed and further investigations showed that the isoelectric process could be adjusted to bring trypsin inhibitor levels of the experimental product down to the levels found in commercial isolates - ultrafiltration process samples however showed higher levels of trypsin inhibitor activity and attempts to reduce these levels adversely affected process yields. Results for phytate were largely inconclusive and attempts to determine lysinoalanine content were unsuccessful due to problems with the analytical procedure.

Amino acid analysis of the experimental samples gave results which correlated well with existing published data. No major differences between the experimental samples were observed.

Polyacrylamide gel electrophoresis of the experimental samples and commercial soya isolates gave significant results in the area of trypsin inhibitor activity. Findings in this area correlated well with those obtained by chemical analysis.

A preliminary study of the comparative economics of the isoelectric and ultrafiltration processes for the manufacture of soya protein isolate was undertaken, using data obtained during the course of the study. Initial findings indicated that the isoelectric process showed a slightly economic advantage, due mainly to the high cost of commercial ultrafiltration plants, however considerable scope exists for reducing the cost of the ultrafiltration process to a level below that of the isoelectric process. The overall viability of soya protein isolate manufacture in South Africa is questionable - current raw material costs appear to be too high for it to be an economic proposition in relation to the currently available imported products.

Considerable scope for further study exists, particularly into process efficiencies for the extraction, centrifugation and ultrafiltration processes. Other areas for investigation would be utilisation of the very large volumes of by-products from the process and the handling of process effluent. A more comprehensive study of these areas might enable the economics of soya isolate production in South Africa to be reviewed, to the potential benefit of both local soya processors and end users, together with the obvious beneficial effect in terms of foreign exchange requirements.

APPENDIX

METHOD FOR DETERMINATION OF SOLIDS CONTENT

PROCEDURE

1. Heat an aluminium sample dish to constant weight then allow to cool in a dessicator.
2. Place 5g accurately weighed sample in the dish.
3. Place dish and sample in 110°C oven overnight.
4. Remove dish and sample from oven, allow to cool in a dessicator and accurately determine the weight of the dish and dried sample.

CALCULATION

$$\% \text{ solids of sample} = \frac{\text{Final dry weight of dish + sample - Initial wt of empty dish}}{\text{sample weight}} \times 100\%$$

METHOD FOR DETERMINATION OF PROTEIN USING BUCHI KJELDAHL APPARATUS.

REAGENTS

1. Concentrated sulphuric acid.

2. 53% sodium hydroxide solution. Dissolve 1650g sodium hydroxide in three litres of distilled water.

3. Catalyst mixture. Mix 14.3g potassium sulphate, 0.6g copper sulphate and 0.07g mercuric oxide.

4. 4.0% boric acid indicator solution. Dissolve 120g boric acid in three litres distilled water and filter.
   To this solution add 42ml methyl red solution (0.1% in alcohol) and 48ml methylene blue solution (0.05% in water)

5. O.1N sulphuric acid (standardised).

PROCEDURE

1. Pre heat Buchi digestion unit.
2. Place sample (sample weight dependent on protein content), 22.5ml concentrated sulphuric acid and 15g of catalyst in a Buchi digestion tube.
3. Fix digestion tube to extraction manifold, plug manifold air entry with cotton wool and place tube in digestion heating unit. Start air suction from manifold.

4. Digest sample until clear digest is obtained then remove tube from digestion unit and allow to cool.
5. Disconnect tube from manifold and place in distillation unit.
6. Add 25ml distilled water to digestion tube contents.
7. Place receiving flask containing 50ml boric acid solution under distillate outlet then add 53% sodium hydroxide to digestion tube until contents of digest tube have become dark in colour with gelatinous precipitate present.
8. Steam distill digestion tube contents until 200ml distillate have been collected in the receiving flask.
9. Discard contents of digestion tube.
10. Titiate contents of receiving flask with 0.IN sulphuric acid.

CALCULATION

$$\% \text{ Protein} = \frac{\text{Titre x normality of Sulphuric Acid x } 1.4 \text{ x } 6.25^{*}}{\text{Wt of sample.}}$$

* The factor of 6.25 was arbitrarily chosen for this study.

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