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Ultrafiltration Studies of Foods: Part 1— The Removal of Undesirable Components in Soymilk and the Effects on the Quality of the Spray-dried Powder

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ABSTRACT

The use of ultrafiltration for the removal of low molecular weight anti-nutritional factors, in particular the oligosaccharides, raffinose and stachyose, and phytic acid, was re-examined. Using a membrane having a 20 000 molecular weight cut-off, the rejection rates of the oligosaccharides and phytic acid increased during ultrafiltration. At 60% water removal over 80% of each of the oligosaccharides was removed. In the case of phytic acid, however, only 50% could be removed. This could be because phytic acid exists as phytates or associated with native protein by salt linkages, and thus complete, or near complete, removal of this would be difficult to achieve even if multiple-stage ultrafiltration (UF) is employed. As would be expected, considerable amounts (50%) of the acid are detected in the soybean soak water. Thus, the actual amount of phytic acid present in the UF concentrate is only about one-third that originally present in the bean. Spray-drying studies were also carried out on UF

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soymilk concentrates. The nitrogen solubility index (NSI) of the spray-dried powder improved with per cent water removal during UF and also by the addition of sucrose to the concentrate prior to spray drying. There is also very little, if any, detectable difference in the taste and flavour.

INTRODUCTION

Reverse osmosis (RO) and ultrafiltration (UF) are relatively new hydraulic pressure-operated membrane techniques in the food industry, particularly in soymilk processing. Their use as a concentration step in industrial unit operations is fast becoming an attractive alternative to conventional evaporation processes as it is a non-thermal treatment, as well as one with mild operating conditions.

Soybean has been a part of the indigenous diet in the Far East for centuries. There is now an increasing emphasis on its use as an important source of low cost protein supplementation to animal protein, as the latter will become more and more expensive due to increasing scarcity. However, it has long been known that soybean contains a number of low molecular weight anti-nutritional factors. The low molecular weight oligosaccharides, raffinose (1–2%) and stachyose (4–8%) (Smith & Circle, 1978; Cegla & Bell, 1977) have been implicated as causative factors in digestive disturbances, such as flatulence (Steggerda *et al.*, 1966; Rackis *et al.*, 1970; Cristofaro *et al.*, 1970, 1974) while phytic acid (myoinositol 1,2,3,5/4,6-hexakis(dihydrogen phosphate)) (1·8%) has been considered to be an anti-nutritional agent that lowers the mineral biodegradability by forming insoluble complexes with di- and tri-valent ions at neutral pH (Cheryan, 1980). All these components cannot be destroyed by heat treatment, like trypsin inhibitors and lipoxygenase. The present work describes the applications of UF in the removal of anti-nutritional low molecular weight components in soymilk processing.

Spray drying has long been the most desirable method for the production of dried milk products. A dry powder product is highly desirable since it not only possesses long shelf-life, but also requires relatively less transportation cost and storage capacity. Thus, a process for producing a dried soymilk powder that is soluble and without loss of nutritive value is highly desirable. The effects of spray drying on the protein profile, amino acids composition, carbohydrate level and nitrogen solubility index (NSI) of soymilk powder obtained by spray drying of soymilk (Aminlari *et al.*, 1977) were studied.

MATERIALS AND METHODS

Preparation of soymilk

Dehulled and cracked Canadian wholebeans were soaked in water overnight and, after draining and rinsing, ground with ten times their weight of water. The resultant slurry was passed into a Fryma toothed colloid mill which will cause further extraction to take place and also serve as a homogenisation step. The slurry was then filtered via a cheese-clothed centrifugal clarifier. All steps were performed at room temperature. The filtered milk was then cooked at approximately 94 °C for 15 min. Efficient stirring was ensured to prevent the formation of burnt flavour.

Batch process of UF soymilk concentrate

The cooked soymilk was then filtered through a cheese cloth and applied to a DDS Lab-Unit 20 UF/HF Module, which used ten stacks of plates sandwiched by membranes, giving an effective membrane surface area of 0·36 m^2. A non-cellulosic membrane (DDS GR 61 P) of 20 000 molecular weight cut-off was used in all the UF experiments.

A series of experiments were carried out at 35 ± 1 °C and the soymilks were concentrated to different solids contents, the highest removed being 60 % v/v water. In all cases, an inlet pressure of 10 bar was used.

During each run the concentrate was allowed to recirculate until the built up pressure within the UF system became a critical factor in governing the permeate flux. This phenomenon will be discussed in a later section.

Spray drying of soymilk concentrate

Sucrose (10 %) and lecithin (0·2 %) were added to the soymilk concentrate and spray-dried using a A/S Niro Atomiser, with an atomiser speed of 24 000 rpm in a concurrent air flow system. The operating conditions were: inlet air temperature, 150 ± 10 °C; outlet air temperature, 65 ± 5 °C.

Analytical procedures

Proximate analyses for moisture, Total Solids and ash were carried out using AOAC methods (AOAC, 1975). Total protein content was

determined using the semi-micro Kjeldahl method (Oser, 1965) and expressed as total nitrogen × 6·25. Fat content was determined by the Majonnier method (Pearson, 1975). Total carbohydrate content was determined by difference since colorimetric measurement using glucose as a reference was found to underestimate the total carbohydrate in soyflour and UF samples (Nicols & Cheryan, 1981). Raffinose, stachyose and phytic acid were quantitatively determined by HPLC (see below). For this work a Perkin-Elmer Series 4 Liquid Chromatography System was used. This comprises a Series 4 Pump Module, an LC-25 Differential Refractometer and a PE 3600 Data Station. The 8 μl flow cell has a standard 0·004 in gasket thickness.

The NSI method, which determines the dispersible nitrogen in the spray-dried product, was based on the AOCS BA11-65 method (AOCS, 1978).

The amino acid compositions of the soymilk, soymilk concentrate and permeate, reconstituted soymilk from spray-dried powder and defatted soymeal were determined using the Technicon NC-2P Autoanalyser.

Determination of oligosaccharides by HPLC

For soymilk

Ten millilitres of the sample were extracted with 20 ml of absolute ethanol by vigorous mechanical agitation at room temperature for 30 min. The resultant slurry was then centrifuged at 2000 rpm for 20 min and the supernatant liquid decanted. The residue was further extracted by stirring with ethanol/water (2:1 v/v), centrifuging and collecting the supernatant. The combined supernatant was then concentrated to dryness at 50 °C under reduced pressure using a Bücchi Rotavapor. The residue was taken up in 2 ml of deionised water, filtered through a 0·45 μm millipore filter. Twenty microlitres of the filtrate were then applied to the PE Li Chromosorb 10 NH_2 column (25 × 0·25 mm) for HPLC analysis using water/acetonitrile (30:70 v/v) as the mobile phase at a flow rate of 1·5 ml/min.

For defatted soymeal

About 1 g of the defatted meal was suspended in 10 ml of deionised water and extracted as described above.

Determination of phytic acid by HPLC

For soymilk

Phytic acid was determined by a modified method of Graf & Dintzis (1982). Five millilitres of the sample were pipetted into a 250-ml conical flask containing 10 ml of water and 5 ml of 2M HCl. The sample was extracted by vigorous mechanical agitation for 2 h at room temperature. The resultant slurry was centrifuged at 2000 rpm for 10 min, filtered through a No. 1 filter paper and then through a 0·45 millipore filter. Ten millilitres of the filtrate were diluted to 40 ml and percolated over an ion-exchange resin column containing 0·5 g Ag 1-8X (Cl^-) (200–400 mesh) anion-exchange resin in 0·8 ml deionised water. The column was then washed with two 10-ml aliquots of 0·1M HCl. Phytic acid was then eluted with 25 ml of 2M HCl at a rate of 0·5 ml/min. Two millilitres of the eluate were concentrated to dryness under reduced pressure in a vacuum desiccator and the residue dissolved in 2 ml of 5 mM sodium acetate solution. Fifty microlitres of the filtered solution (through a 0·45 μm millipore filter) were applied to the PE/Analytical C18 (25 cm × 2·6 mm) column and eluted with 5 mM sodium acetate solution at a flow rate of 1 ml/min. The eluted phytic acid was quantitatively determined using the PE-LC25 refractive index detector.

For defatted soymeal

Two grams of the soymeal were suspended in 40 ml of 0·5 M HCl and extracted as described above.

The modified method used gave a 93% recovery rate of phytic acid as compared with the 76% reported by Graf & Dintzis (1982).

RESULTS AND DISCUSSION

Soybean hulls contain water-soluble pigments, including anthocyanins, which may interact with protein during preparation of soymilk extract (Smith & Circle, 1978). Cracked and dehulled wholebean was therefore used as the starting raw material in our study. Cooking of the soymilk extract is essential as it imparts an acceptable flavour, as well as inactivating lipoxygenase and inhibiting the activity of trypsin inhibitor.

The presence of high molecular weight solute or colloid and insoluble

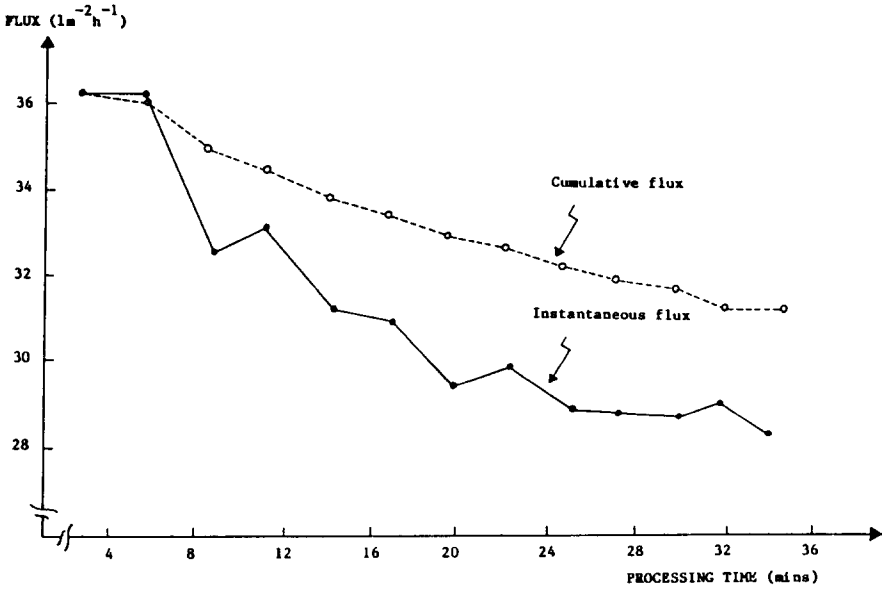

Fig. 1. Variation of flux with processing time.

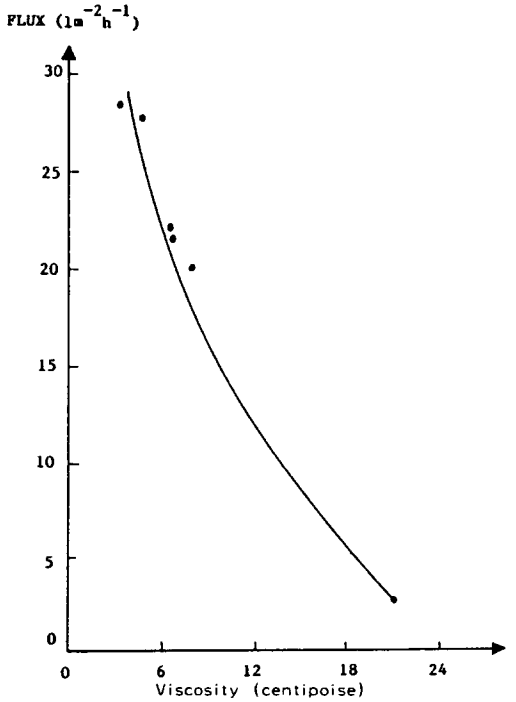

Fig. 2. Plot of flux versus function of viscosity.

foreign matter will result in solute accumulation at the membrane surface during ultrafiltration and produces a layer of finite, and frequently large, hydraulic flow resistance (Delaney *et al.*, 1973) and thereby decreases the overall hydraulic permeability of the membrane. Thus, careful filtration of the extract is essential for optimum ultrafiltration performance. Figure 1 shows a typical decline in permeate flux with time. The initial flux was approximately 36 litres m^{-2} h^{-1}. The permeate flux dropped very steeply at high viscosity and reached an unacceptable level at a viscosity value of 20 cP; the initial viscosity of the feed was 4 cP (Fig. 2). Work using a 50 000 molecular weight cut-off is now being tried and preliminary results indicate that higher per cent water removal may be possible.

The spray dryer outlet temperature of 65 ± 5 °C was found to cause least wall deposition problems. Higher outlet temperatures were found to reduce the NSI value of the spray-dried powder considerably. Lecithin was added to the feed for spray drying to improve the wetting time, as well as to act as an antioxidant.

Composition of UF concentrate and permeate

Table 1 shows the composition of the different soymilk samples analysed whilst Figs 3 and 4 show the changes of these soy components with per

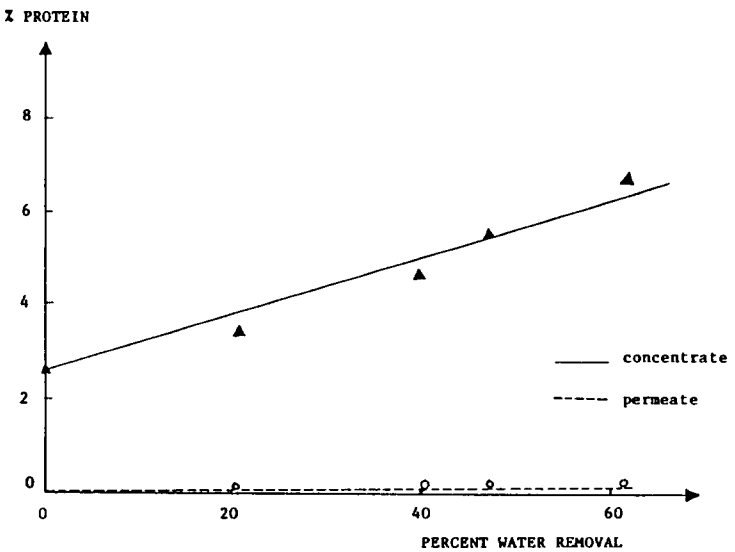

Fig. 3. Change in protein content with water removal during UF.

TABLE 1
Analysis of Soymilk Samples

Analysis (% w/v)	*Soymilk from defatted soy meal*[b]	*Ultrafiltration of soymilk*[a]*: per cent water removal*						
		0% water removal	*20% water removal*		*40% water removal*		*60% water removal*	
			Conc.	*Permeate*	*Conc.*	*Permeate*	*Conc.*	*Permeate*
Total solids	5·70	4·83	5·82	0·23	7·54	0·36	10·68	0·54
Ash	0·31	0·28	0·30	0·02	0·33	0·06	0·39	0·08
Protein (N × 6·25)	2·50	2·60	3·28	0·05	4·32	0·07	5·62	0·09
Fat	1·30	1·55	1·80	0·05	2·15	0·06	2·64	0·08

[a] Dehulled soybean:water ratio, 1:10.
[b] Ang *et al.* (1985).

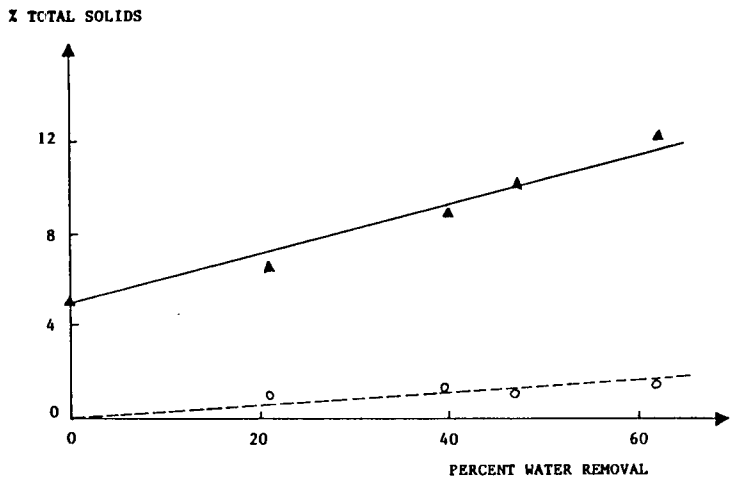

Fig. 4. Change in Total Solids with water removal during UF.

cent water removal. It can be seen that the UF concentrate exhibits a linear increase in Total Solids, fat and protein content. It is to be expected that, for a component that is completely rejected by a membrane, an x-volume reduction would cause an x-fold decrease in the concentration of that component. However, it can be seen that for the 40 % and 60 % water removal, the increases in these components far exceed 40 % and 60 %, respectively, clearly indicating that some low molecular weight components in the soymilk have been lost during ultrafiltration.

The major proteins of soybean have molecular weights ranging from 200 000 to 600 000. The use of 20 000 molecular weight cut-off membranes will allow only peptides of molecular weight less than this value to pass through the membrane. The low permeate protein content (Table 1, Fig. 3) reflects a protein retention close to 100 %.

Fat globules are also larger than the pore size of the membrane and are thus expected to be retained by the membrane. Table 1 shows that only insignificant quantities of fat were detected in the permeates.

Because of the small molecular size of inorganic salts, these would be expected to pass through the membrane. Table 1 and Fig. 5 show the increase in ash content with increase in per cent water removal. At 20 % water removal, 7 % of inorganic matter was found in the permeate while at 40 % and 60 % removal these values were 21 % and 38 %, respectively. This is also reflected in the spray-dried product (Table 2). This is probably because of solute–solute interaction. The minerals become bound to the

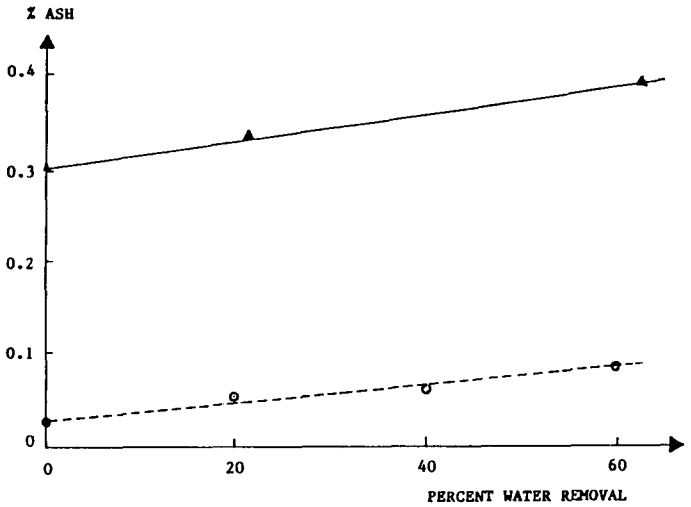

Fig. 5. Change in ash content with water removal during UF.

protein as the solution is being concentrated. This has been suggested by Osmosaiye & Cheryan (1979) and Nicols & Cheryan (1981). This mineral–protein interaction may probably account for the sudden increase in viscosity during ultrafiltration. Above 60% water removal, the hydraulic flow resistance in the boundary layer becomes too great, thus reducing the effective pressure for ultrafiltration.

TABLE 2
Analysis of Soymilk Powder Obtained by Spray Drying UF Soymilk Concentrate[a]

Analysis (% *w/w*)	*Spray-dried soymilk powder from UF concentrate*				*Soybean soak water*
	0% water	*20% water*	*40% water*	*60% water*	
Moisture	1·45	1·74	2·01	2·17	—
Ash	1·42	1·46	1·49	1·76	—
Protein (N × 6·25)	15·60	18·60	22·60	27·21	—
Fat	9·30	10·95	12·98	15·18	—
Phytic acid	18·00	14·00	11·00	9·00	9·00
Raffinose	0·92	0·70	0·50	0·16	0·16
Stachyose	3·20	2·60	1·60	0·56	0·92
Sucrose[b]	5·94	4·90	2·90	0·85	1·06

[a] Soymilk concentrate contains 10% added sucrose.
[b] No added sucrose in the soymilk concentrate.

TABLE 3

Amino Acids Composition (mg/ml) of UF Soymilk Concentrate and Permeate Using GR 61 P Membrane (20 000 Molecular Weight Cut-off)

Amino acid	*Soymilk samples*						
	0% water removal	*20% water removal*		*40% water removal*		*60% water removal*	
		Conc.	*Peremeate*	*Conc.*	*Permeate*	*Conc.*	*Permeate*
Aspartic acid	2·20	2·79	0·10	3·22	0·07	4·45	0·03
Threonine	0·71	0·80	0·06	0·96	0·05	1·50	0·01
Serine	0·74	0·86	0·01	1·04	ND	1·79	ND
Glutamic acid	3·24	4·15	0·14	4·66	0·12	6·14	0·04
Proline	1·14	1·05	ND	1·33	ND	2·31	ND
Glycine	0·76	0·98	0·09	1·16	0·07	1·30	0·03
Alanine	0·80	0·97	0·04	1·20	0·03	1·80	0·01
Cystine	ND	ND	ND	ND	ND	0·99	ND
Valine	0·60	0·84	0·04	0·93	ND	1·40	ND
Methionine	Trace	Trace	ND	Trace	ND	0·44	ND
Isolucine	0·82	1·02	0·04	1·15	0·04	1·14	0·01
Leucine	1·43	1·63	ND	2·79	ND	2·83	0·01
Norleucine	ND	ND	ND	ND	ND	ND	ND
Tyrosine	0·87	1·02	0·04	1·18	ND	1·33	ND
Phenylalanine	0·88	1·24	ND	1·46	ND	1·73	ND
Histidine	0·53	0·63	0·03	0·86	ND	0·84	ND
Lysine	1·23	1·43	0·09	1·92	0·04	2·29	0·04
Arginine	1·13	1·44	0·11	1·76	0·09	2·32	ND

ND, not determined.

TABLE 4
Amino Acids Composition (mg/g) of Spray-Dried Powder from UF Soymilk Concentrate

Amino acid (mg/g)	*Soymilk samples*			
	0% removal[a]	*20% removal*	*40% removal*	*60% removal*
Aspartic acid	15·50	19·00	24·1	25·0
Threonine	4·80	5·92	6·88	8·22
Serine	7·70	8·06	8·39	7·74
Glutamic acid	27·1	28·56	36·9	37·6
Proline	9·00	15·9	10·65	14·5
Glycine	5·00	5·78	7·85	9·80
Alanine	5·60	7·86	9·79	11·2
Cystine	Trace	Trace	Trace	Trace
Valine	5·60	6·40	7·36	10·6
Methionine	3·70	2·89	2·84	2·60
Isoleucine	6·00	4·80	8·24	10·3
Leucine	10·3	9·90	12·4	13·7
Norleucine	ND	ND	ND	ND
Tyrosine	5·00	4·28	6·83	6·08
Phenylalanine	6·40	8·64	11·1	10·9
Histidine	4·6	1·42	1·21	8·26
Lysine	11·0	8·32	11·4	15·8
Arginine	11·0	10·2	10·4	15·0

[a] Ang *et al.* (1985).
ND, not determined.

Amino acids composition

The amino acids composition of soymilk, the UF concentrates and permeates, and the spray-dried powders are shown in Tables 3 and 4. These show that the amino acids contents increase nearly linearly with the per cent water removal. In each case negligible amounts of amino acids are detected in the permeates, thus confirming the near complete retention of the proteins during ultrafiltration.

Removal of oligosaccharides by UF

Osmosaiye *et al.* (1978) have reported that rejection of oligosaccharides from soymilk extract during ultrafiltration using an HF 15-45-XM50 hollow fibre cartridge (molecular weight cut-off of 50 000) remained constant. However, they found that 71–80% of the oligosaccharides

(including sucrose) could be removed by processing to a volume concentration ratio of 5 and, with two such ultrafiltration steps, as much as 96% could be removed. We have also obtained similar results. Analysis of powder obtained by spray drying the 60% water removal UF concentrate shows that over 80% rejection of each of the oligosaccharides, raffinose and stachyose, content has occurred. Analysis of sucrose was also carried out on spray-dried powder from soymilk which did not contain the 10% added sucrose. The powder obtained from the soymilk concentrate in which 60% water had been removed showed rejection of over 85% sucrose (Table 2).

As reported by Kim *et al.* (1973), Ku *et al.* (1976) and Osmosaiye *et al.* (1978), we have also found that significant amounts (*ca.* 20%) of oligosaccharides were leached into the soak water. However, it is difficult to compare these results with those of the other workers since our bean:soak water ratios were different from theirs.

Removal of phytic acid by UF

The HPLC analyses of a series of 60% water removal concentrates (Table 2) establish that only about 50% of the phytic acid present in soymilk extract was rejected during ultrafiltration. Okubo *et al.* (1975), however, reported an 80% removal but their procedure involved numerous steps, including the use of low pH to bring about dissociation of the phytates, followed by a two-step ultrafiltration (diafiltration). Since phytic acid exists as phytates and as the phytates in soybeans are known to be associated with part of the native protein by salt linkage (McKinney *et al.*, 1949), complete, or nearly complete, removal from soymilk will be difficult to achieve. Methods which might be used for removal of the phytates from protein are available (McKinney *et al.*, 1949) but the time required is too long for a commercial operation.

Being water-soluble, phytic acid would be expected to be leached into the soak water. Examination of the soak water (Table 2) showed that about 50% had been leached in this way. Thus, the actual amount of phytic acid in the soymilk and the spray-dried powder is only about 33% of its original value.

Characteristics of the product

Table 5 shows the relationship between per cent water removal, protein content and the nitrogen solubility index (NSI) of spray-dried powder.

TABLE 5
Protein and NSI Value of Spray-Dried Powder After Ultrafiltration

Spray-dried powder	*Protein (% w/w)*	*NSI (%)*
Control[a]	—	52·0
0% water removal	15·6	87·0
20% water removal	18·6	95·0
40% water removal	22·6	97·0
60% water removal	27·2	98·8

[a] Control: soymilk is not subjected to UF and does not contain added sucrose and lecithin.

The protein content increases from 16% to 27% following the removal of 60% water (Fig. 6). This is accounted for from the loss of low molecular weight components during the filtration process.

Factors that contribute to reconstitutability are both physical and chemical, such as the composition, especially, ratio of solids-not-fat to fat of the particles, the presence of additives and the amount of protein denaturation by heat (Hall & Hedrick, 1966). Thus, the property of dry milk reconstitutability is affected by the equipment and processing conditions: the drier and system of atomization, heat stability of the milk, preheat treatment of milk and concentrate, Total Solids of the

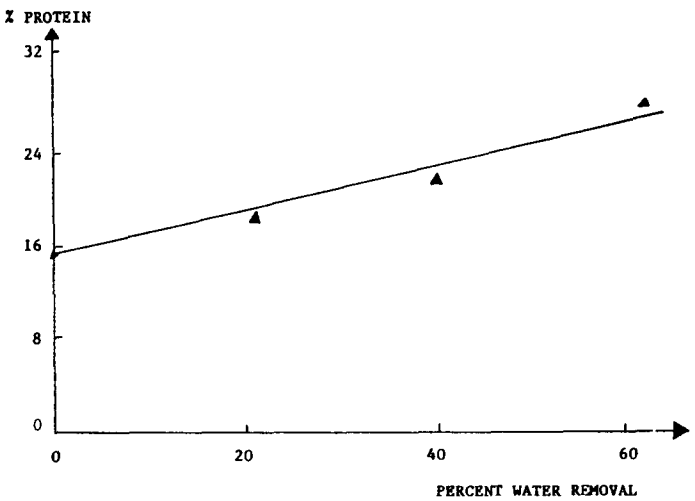

Fig. 6. Protein content of spray-dried powder obtained from UF soy milk concentrate.

concentrate and outlet air temperature and contact time. For good dispersibility an important consideration is the total heat treatment on the protein during processing. In general, increased heat application with increasing Total Solids causes larger amounts of irreversible denaturation. For air- or vacuum-dried defatted soy flakes, Beckel and coworkers (Beckel *et al.*, 1946) reported that nitrogen extracted by water is at a maximum at about 70–75 °C. The temperature effect is greater for heat-treated flakes, the maximum being about 62 °C (Circle *et al.*, 1959). The spray-drying conditions employed, however, do not normally cause significant denaturation unless attempts are made to obtain production above the normal capacity of the drier. The outlet temperature of 65 ± 5 °C was found to be the best temperature for least wall deposition and maximum nitrogen extraction.

Large particle size of dry milk is generally recognised as necessary for good dispersability (Hall & Hedrick, 1966) since larger particles, more irregular in shape, would provide more space in the interstices for wetting. It was observed that spray-dried soymilk containing 10 % added sucrose gave products which have larger particle size than those without added sucrose. Furthermore, these products are slightly hygroscopic and tend to aggregate and form larger particles with larger spaces among them as a result of agglomeration, giving the improved NSI value (Table 5). This increased NSI value over that which did not contain added sucrose and was not subjected to UF treatment, is from 52 % to 87·2 % (Table 5). The value increases with increase in water removal, reaching 98 % for a 50 % concentration, and the product was virtually completely soluble at 60 % concentration. This phenomenon has been reported in drying of cow's milk. The addition of up to 25 % sugars to the milk before drying, or by dry blending the sugar in the granulated form, improves wettability and dispersion (Hall & Hedrick, 1966).

The amount and dispersion of fat should also affect wettability, since it is hydrophobic. There may probably be some inhibition of wetting in relation to the amount of fat present but the test is not sensitive enough to detect the difference in small increments.

It is interesting to note that it was not possible to extract the fat from the spray-dried powder containing 10 % sucrose using the Soxhlet method. No problem was, however, encountered with a reconstituted sample, using the Majonnier method. It thus seems to appear that the fat is encapsulated in some way by the added sugar, thus preventing the ether/petroleum ether from solubilising it.

CONCLUSION

The composition of the ultrafiltrated soymilk concentrates revealed that the protein and amino acids contents have been enriched linearly with the percentages of water removal. Furthermore, the undesirable components that are heat-resistant, such as raffinose, stachyose and phytic acid, have been significantly reduced. Complete removal would, however, be very difficult and expensive, but not impossible. Being water-soluble, substantial amounts of these would be expected and have been found in the soak water. The final spray-dried product, in terms of protein and amino acids contents, raffinose and stachyose, and phytates, is nutritionally superior to the original milk. Its dispersibility in water on reconstitution, as measured by the NSI value, is very high and there is very little, if any, detectable difference in flavour.

REFERENCES

Ang, H. G., Kwik, W. L. & Theng, C. Y. (1985). Development of soymilk. *Food Chem.*, **17**, 235–50.

AOAC (1975). *Official methods of analysis* (12th edn), Association of Official analytical Chemists, Washington, DC.

AOCS (1978). *Official and Tentative Methods of the American Oil Chemists' Society* (3rd edn), Champaign, Illinois.

Aminlari, M., Ferrier, L. K. & Nelson, A. I. (1977). Protein dispersibility of spray dried whole bean milk base: Effect of processing variables. *J. Food Sci.*, **42**, 985–88.

Beckel, A. C., Belter, P. A. & Smith, A. K. (1946). Soybean protein production. Effect of temperature and water:flake ratio. *Ind. Eng. Chem.*, **38**, 731–34.

Cegla, G. F. & Bell, K. R. (1977). High pressure liquid chromatography for the analysis of soluble carbohydrates in defatted oilseed flours. *J. Amer. Chem. Oil Soc.*, **54**, 150–54.

Cheryan, M. (1980). Phytic acid interactions in food system. *Crit. Rev. Food Sci. Nutr.*, **14**, 297–335.

Circle, S. J., Julian, P. L. & Whitney, R. W. (1959). Process for isolating soya protein. US Pat., 2,881,159.

Cristofaro, E., Mottu, F. & Wuhrmann, J. J. (1970). Study of the effect of stachyose and raffinose on the flatulence activity of soymilk. Paper presented at the 3rd International Congress of Food Science and Technology, August 9–14, Washington, DC.

Cristofaro, E., Mottu, F. & Wuhrmann, J. J. (1974). Involvement of the raffinose family of oligosaccharides in flatulence. In: *Sugars in nutrition* (Supple, H. L. & McNutt, K. W. (Eds)), Academic Press, New York, 313–36.

Delaney, R. A. M., Donnelly, J. K. & O'Sullivan, A. C. (1973). Lactose and reverse osmosis. In: *Molecular structure and function of food carbohydrate* (Birch, G. G. & Green, L. F. (Eds)), Applied Science Publishers, London, pp. 155–75.

Graf, E. & Dintzis, F. R. (1982). Determination of phytic acid in foods by high-performance liquid chromatography. *J. Agric. Food Chem.*, **30**, 1094–97.

Hall, C. W. & Hedrick, T. I. (1966). *Drying milk and milk products*, AVI, Westport, Conn.

Kim, W. J., Smit, C. J. B. & Nakayama, T. O. M. (1973). The removal of oligosaccharides from soybeans. *Lebensm.-Wiss. u. Technol.*, **6**, 201–4.

Ku, S., Wei, L. S., Steinberg, M. P., Nelson, A. I. & Hymowitz, T. (1976). Extraction of oligosaccharides during cooking of whole soybeans. *J. Food Sci.*, **41**, 361–64.

McKinney, L. L., Sollars, W. F. & Setzkorn, E. A. (1949). Studies on the preparation of soybean protein free from phosphorus. *J. Biol. Chem.*, **178**, 117–32.

Nicols, D. J. & Cheryan, M. (1981). Production of soy isolates by ultrafiltration: Factors affecting yield and composition. *J. Food Sci.*, **46**, 367–72.

Okubo, K., Waldrop, A. B., Iacobucci, G. A. & Myers, D. V. (1975). Preparation of low phytate soybean protein isolate and concentrate by ultrafiltration. *Cereal Chem.*, **52**, 263–69.

Oser, B. L. (1965). *Hawk's physiological chemistry* (14th edn), McGraw-Hill, New York.

Osmosaiye, O. & Cheryan, M. (1979). Ultrafiltration of soybean water extracts: Processing characteristics and yield. *J. Food Sci.*, **44**, 1027–31.

Osmosaiye, O., Cheryan, M. & Mathew, M. E. (1978). Removal of oligosaccharides from soybean water extract by ultrafiltration. *J. Food Sci.*, **43**, 354–60.

Pearson, D. (1975). *Chemical analysis of foods* (7th edn), Longman, UK.

Rackis, J. J., Sessa, D. J., Steggerda, F. R., Shimizu, J., Anderson, J. & Pearl, S. L. (1970). Soybean factor relating to gas production by intestinal bacteria. *J. Food Sci.*, **35**, 634–39.

Smith, A. K. & Circle, S. J. (1978). *Soybeans: Chemistry and technology*, Vol. 1. AVI, Westport.

Steggerda, F. R., Richards, E. A. & Rackis, J. J. (1966). Effects of various soybean products on flatulence in the adult man. *Proc. Soc. Exp. Biol. Med.*, **121**, 1235–39.