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Emulsifying properties of soy protein isolates obtained by microfiltration

Bernard E Chove, Alistair S Grandison* and Michael J Lewis
School of Food Biosciences, The University of Reading, PO Box 226 Whiteknights, Reading RG6 6AP, UK

Abstract: Soy protein isolate (SPI) fractions were produced using two different pore size microfiltration membranes. Microfiltration was carried out on SPI produced by isoelectric precipitation of a crude protein extract. Five fractions were obtained: two retentates and two permeates from the two membranes plus an intermediate fraction obtained as the retentate on the small-pore-size membrane using the permeate from the larger-pore-size membrane. Emulsions stabilised by the retentate fractions exhibited higher values ($P < 0.01$) of emulsion stability index (ESI) and emulsifying activity index (EAI) than those stabilised with fractions made from the permeates. The intermediate fraction gave intermediate ESI values, while the EAI values were not significantly different from those for SPI and one of the retentates. SDS-PAGE profiles indicated that the fractions exhibiting high functionality in terms of ESI and EAI were also richer in 7S globulin soy protein subunits.

Keywords: soy protein; isolate; microfiltration; membranes; fractionation; isoelectric focusing; emulsifying activity; emulsion stability

INTRODUCTION

The present trend towards complete food formulation from refined ingredients has resulted in a growing demand for food grade proteins. Traditional animal sources of protein, although nutritionally and functionally superior, cannot continue to adequately meet demand because of their cost and limited supply.[1] Vegetable protein is the most abundant source of protein on earth, and numerous vegetables have been investigated for possible incorporation into formulated foods.[2] Soy proteins are used in foods as functional and nutritional ingredients, but in their native form they lack the required functionality. Critical functional properties necessary in protein ingredients include solubility, water and fat absorption, emulsion stabilisation, whippability, gelation and good organoleptic properties.[3]

Many physical, chemical and enzymatic modifications have been used to expand the range of functional properties in soy proteins.[2,4,5] These modifications can be costly in terms of the process itself as well as losing some other properties at the expense of improving the targeted ones. Chemical modification also poses the problem of removing any unreacted reagents from the final product.[6]

Ultrafiltration membranes have been applied for recovery of the whole soy protein isolate.[7,8] Membrane processes have been shown to offer economic advantages by improving yield and functionality of the isolate and eliminating major waste treatment problems.[9] Soy protein produced solely by membrane processing is very soluble owing to the elimination of acid precipitation and neutralisation steps responsible for the loss of solubility, but the yield is limited.[10] Soy protein fractionation by a combination of ultrafiltration and some chemical treatments has also been reported,[11–13] but the methods are mainly for the small-scale production of analytical grade products. These procedures also involve further pH manipulations as well as the conventional isoelectric precipitation method. This results in further loss of functionality of the separated fractions, as noted above.

The aim of the present work was to fractionate the conventionally extracted soy protein isolate (SPI) by using microfiltration membranes, and to assess the functionality of the resulting protein fractions. SDS-PAGE was employed to profile the molecular weights, and isoelectric focusing was also carried out in an attempt to relate the functional properties to the charge carried by individual molecular species in the fractions. Fundamental knowledge about emulsifying properties of soy proteins is scanty in comparison with their other properties such as gelling and foaming.[14] Two emulsification functional properties were used as tests for functionality: the emulsion stability index (ESI) and the emulsification activity index (EAI).

MATERIALS AND METHODS

Materials

Defatted soy flour supplied by Food Ingredient Technology Limited (Great Gransdon, UK) was used

* Correspondence to: Alistair S Grandison, School of Food Biosciences, The University of Reading, PO Box 226, Whiteknights, Reading RG6 6AP, UK
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as the starting material. The protein dispersibility index (PDI) was specified as 80%. Analytical reagent grade chemicals were supplied by Sigma-Aldrich Co Ltd (Poole, UK).

Methods

Soy protein isolate (SPI) preparation

Defatted soy flour was dispersed in distilled water at 22 °C (10% w/v) and the pH was adjusted to 9.0 (using 1.0 M NaOH) to solubilise the proteins.[4] The suspension was stirred using a magnetic stirrer for 2.5 h. The insoluble solid was separated out by centrifugation (Model RC 5C plus, Sorvall Products, Newtown, USA) at $10\,000 \times g$ for 25 min. The pH of the aqueous extract (crude protein extract) was lowered to 4.5 (the isoelectric point of soy proteins) by adding 1.0 M HCl. The precipitated protein was separated from the soy whey by centrifugation at $10\,000 \times g$ for 10 min. The precipitate was washed twice by dispersing in deionised water (pH 4.5) and centrifuging at $10\,000 \times g$ for 10 min. The washed precipitate was finally dispersed in deionised water (10% w/v) and the pH adjusted to 7.0 using 1.0 M NaOH. This constituted the main SPI solution.

Fractionation of SPI

Microfiltration was carried out batch-wise using a dead-end stirred cell (Gyrosep™ 300, Techmate Ltd, Milton Keynes, UK). The starting batch volume was 300 ml of the SPI solution for each run, except for the intermediate fraction for which the starting solution was 300 ml of the permeate collected using the large-pore-size membrane. The cell was pressurised at a constant pressure of 3.0 bar throughout the process. This pressure is high for microfiltration, but was necessary to achieve reasonable fluxes in this stirred cell geometry. Two membranes were employed: Sepraphore III (cellulose acetate) with an average pore size of 0.1 μm and Celgard 3500 (polypropylene/polyethylene mixture) with rectangular pores of $0.05\,\mu m \times 0.02\,\mu m$ (Intersep Filtration Systems, Wokingham, UK). A die was used to cut circular discs (0.075 m diameter) from the supplied membrane sheets. Each disc presented an effective filtration area of $3.85 \times 10^{-3}\,m^2$. The Celgard was hydrophilised overnight in absolute isopropanol and carefully washed in plenty of deionised water before use. The filtration process lasted between 1 and 1.5 h before the membrane became fouled, at which point a volume reduction factor of 4.2 had been achieved with the Sepraphore membranes and a factor of 7.5 with the Celgard membranes. Stirring was carried out in reverse period cycle with forward stirring up to a maximum of 350 rpm and reverse stirring up to 90 rpm. The permeate flux rate ranged from a maximum of $2 \times 10^{-5}\,m^3\,s^{-1}\,m^{-2}$ ($0.277\,l\,h^{-1}$) at the beginning to a minimum of $4 \times 10^{-6}\,m^3\,s^{-1}\,m^{-2}$ ($0.05\,l\,h^{-1}$) at the end of the process using the Sepraphore III and Celgard 3500 respectively. Both permeate and retentate from each membrane were collected for analysis. The protein content of the fractions and that of the SPI were determined by the Kjeldahl method,[15] employing a nitrogen-to-protein conversion factor of 6.25.[2] All experimental results are the means of two separate microfiltration experiments, which yielded reproducible results throughout.

Isoelectric focusing

Ready-to-use IsoGel agarose IEF plates (pH 3–10) and the corresponding focusing, fixing, staining and destaining procedures were supplied by FMC Bio Products, (Maine, USA). The pI markers (IEF-MIX 3.6–9.3, Sigma-Aldrich Co, UK) were prepared and applied as described by Righetti and Drysdale.[16] The voltage was set constant at 1100 V and the initial focusing power at 25 W. A horizontal focusing chamber (2117 Multiphor, LKB, Amersham, UK) was used in the process.

SDS-PAGE

The molecular weight profiles for the protein fractions were established using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli.[17]

Protein samples ($1\,\mu g\,\mu l^{-1}$) were prepared in a buffer containing 62.5 mM Tris-HCl (pH 6.8), 2% SDS, 10% glycerol, 5% 2-mercaptoethanol and 0.002% bromophenol. Protein samples (15 μl) were loaded onto the gel. SDS-PAGE was carried out on a slab gel, and the electrode buffer (pH 8.6) contained 25 mM Tris, 192 mM glycine and 0.1% SDS. The separating gel contained 12.5% final acrylamide concentration, while the stacking gel was of 3% concentration. Low- and high-range molecular weight markers (Sigma Markers, Sigma-Aldrich Co, UK) were loaded alongside the samples (5 μm). After staining (brilliant blue R) and destaining, the gels were analysed using the SYNGENE Gel documentation system employing the software program Gene Tools, both supplied by Genetic Research Instrumentation Ltd (Braintree, UK). The approximate molecular weights and quantities (%) of the proteins in each lane were established using the Gene Tools software program.

Preparation of emulsions and oil droplet size determination

Oil-in-water emulsions were prepared using a pressure homogeniser (MFC Microfluidiser Series 5000, Microfluidics Corporation, Newton, USA). Corn oil ($0.25\,l\,l^{-1}$) and protein fraction dispersions ($10\,g\,kg^{-1}$) were mixed and homogenised at 30 MPa. Two passes were used to ensure uniform mixing of the oil and the protein solutions. The oil droplet size distribution was established using a laser diffraction particle size analyser (Malvern Sizer, Series 2600C, Malvern Instruments Ltd, Malvern, UK).

Emulsifying properties

The emulsion stability index (ESI) and emulsifying activity index (EAI) for the protein-stabilised emulsions were determined by turbidimetric methods.[11,18]

Freshly prepared emulsions (1 ml) were pipetted out at 0 and 10 min after homogenisation and serially diluted with 99 ml of distilled water (100-fold) followed by 1 ml of the diluted emulsion into 39 ml (40-fold) of 1 g kg^{-1} SDS (to avoid flocculation), resulting in a 4000-fold total dilution. Absorbance of the final dispersion was measured at 500 nm (Philips PU 8620 UV/VIS/NIR Spectrophotometer, Pye Unicam Ltd, Cambridge, UK). The ESI and EAI were determined as follows.

$$\text{ESI (min)} = \left(\frac{A_0}{\Delta A}\right) t$$

where A_0 is the absorbance of the diluted emulsion immediately after homogenisation, ΔA is the change in absorbance between 0 and 10 min ($A_0 - A_{10}$) and t is the time interval, 10 min in this case.

$$\text{EAI (m}^2\,\text{g}^{-1}) = 2T\left(\frac{A_0 \times \text{ dilution factor}}{C \times \Phi \times 10000}\right)$$

where $T = 2.303$, C is the weight of protein per unit volume (g ml^{-1}) of the protein aqueous phase before emulsion formation, Φ is the oil volume fraction of the emulsion (0.25 in this case) and the dilution factor was 4000.

Statistical analysis

A one-way analysis of variance (ANOVA) using the Minitab 13 for Windows (Version 13.2, Minitab Inc, State College, PA, USA) statistical software program was applied to compare the means of turbidity measurements from four replicates of protein-stabilised emulsions. Significance was defined at $P < 0.01$.

RESULTS AND DISCUSSION

IEF and SDS-PAGE profiles for protein fractions

The separated fractions were analysed for their profiles in terms of charge distribution and size by molecular weight distribution. The isoelectric focusing (IEF) technique was employed in establishing the charge distribution for the molecular species in the protein fractions. Fig 1 presents the IEF profiles for the fractions alongside those for the conventionally extracted soy protein isolate (SPI) and the soy whey protein. There were no noticeable differences between the profile for the SPI and that of the fraction retained on the Sepraphore III (0.1 μm) membrane. This is most probably due to the fact that the retentate comprised the bulk of the protein from the feed material, which was the SPI in this case. However, the permeate from this membrane shows enrichment of the species with pI at approximately 4.6 and 4.7 and depletion of those species with pI above 5.1. The same trend can be observed for the retentate and permeate collected from the Celgard (0.05 μm × 0.02 μm) membrane, except for the fact that the extent of enrichment and depletion was greater in the latter case. The intermediate fraction $FR_{(0.1–0.05)}$ (ie the one collected as retentate on the Celgard from the permeate through the Sepraphore III membrane) exhibited an intermediate composition. The pI 4.6 and 4.7 bands were enriched to a lesser extent compared with the two permeates, while the cluster with pI above 5.1 is partially depleted compared with the two rententates. The whey was shown to have two bands at pI 9.3 and 7.2 which are not exhibited by any of the other fractions. It also contained a cluster of bands between pI 4.0 and 5.1 with one strong band at pI 4.6.

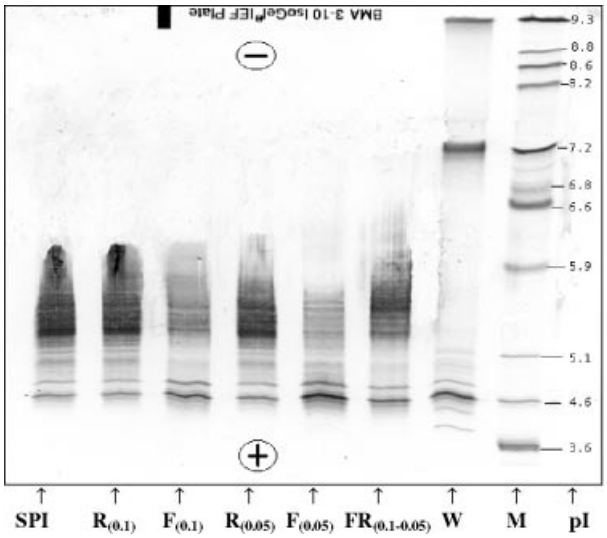

Figure 1. IEF profiles for the separated fractions: SPI, conventional soy protein isolate; $R_{(0.1)}$, retentate from the Sepraphore III (0.1 μm) membrane; $F_{(0.1)}$, permeate through the Sepraphore III (0.1 μm) membrane; $R_{(0.05)}$, retentate from the Celgard (0.05 μm × 0.02 μm) membrane; $F_{(0.05)}$, permeate through the Celgard (0.05 μm × 0.02 μm) membrane; $FR_{(0.1–0.05)}$, retentate from the Celgard (0.05 μm × 0.02 μm) membrane using the Sepraphore III (0.1 μm) membrane permeate; W, soy whey protein; M, pI markers.

The SDS-PAGE profiles for the fractions, alongside those for the crude protein extract, SPI and soy whey, are shown in Fig 2. The quantities of protein in each detectable band (expressed as a percentage of the total protein in all the detectable bands in a given lane) are summarised in Table 1. The crude extract had 11 detectable bands located at approximately 80, 76, 65, 50.1, 35, 33, 28.4, 22, 20, 14.6 and 3.6 kDa.

Some of these can be identified as follows: α', α and β subunits of the 7S globulin at 80, 76 and 50.1 kDa respectively.[19] Two acidic subunits of the 11S globulin are identified at 35 and 33 kDa, and two basic subunits of the 11S globulin at 22 kDa[20] and 20 kDa.[21] As a general observation, the major constituent bands in all the fractions are those at 20 and 35 kDa, followed by the 50.1 kDa band. Indeed, the largest fraction, which accounts for over 40% of the total soybean seed globulin, is the 11S globulin.[22] The profile for the SPI closely follows that of the crude extract in terms of the main constituent bands, as does that for the 0.1 μm membrane retentate, showing slight increases in the percentages of the 80, 76, 41, 35 and 15.6 kDa bands with simultaneous decreases in those at 20 and 14.6 kDa. The 0.05 μm × 0.02 μm membrane retentate

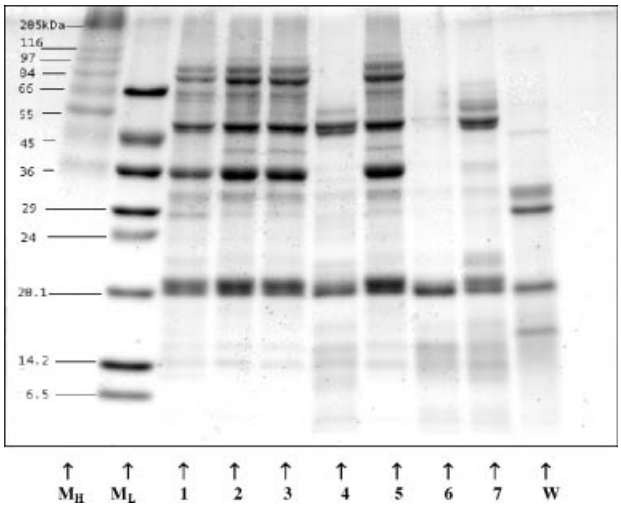

Figure 2. SDS-PAGE profiles for the separated fractions; M_H, high-range molecular weight markers; M_L, low-range molecular weight markers; lane 1, crude extract; lane 2, conventional soy protein isolate; lane 3, retentate from the Sepraphore III (0.1 μm) membrane; lane 4, permeate though the Sepraphore III (0.1 μm) membrane; lane 5, retentate from the Celgard (0.05 μm × 0.02 μm) membrane; lane 6, permeate through the Celgard (0.05 μm × 0.02 μm) membrane; lane 7, retentate from the Celgard (0.05 μm × 0.02 μm) membrane using the Sepraphore III (0.1 μm) membrane permeate; W, soy whey protein.

displayed a similar composition in the high-molecular-weight bands at 50.1, 65, 76 and 80 kDa, but it exhibited traces of the 28.4 and 22 kDa bands, which were not detectable in the SPI and the 0.1 μm membrane retentate. It also demonstrated a slight increase in the 20 kDa band.

The two permeates $F_{(0.1)}$ and $F_{(0.05)}$ were quite different in composition compared with the SPI and the two retentates. They both lacked the high-molecular-weight bands at 65, 76 and 80 kDa and the bands at 35 and 41 kDa. In addition, the $F_{(0.05)}$ fraction also lacked the 22, 28.4 and 33 kDa bands. However, this fraction displayed big increases in the 3.6, 15.6 and 20 kDa bands. The $F_{(0.1)}$ fraction had detectable bands at 60, 28.4 and 22 kDa, while the 20 and 14.6 kDa bands were markedly increased. The intermediate fraction $FR_{(0.1–0.05)}$ also lacked the high-molecular-weight bands at 65, 76 and 80 kDa, but it was enriched in the 60 kDa band, which is identified to be β-amylase.[19] The low-molecular-weight bands at 3.6, 14.6, 15.6 and 20 kDa were also increased compared with those from the retentates.

Emulsifying properties

The indices for emulsifying properties (ESI and EAI) of the emulsions produced using the separated fractions are compared in Fig 3 and 4. From the findings of a previous study,[23] the soy whey was excluded from the present study because it was found to have poor emulsification properties. It can be seen from Fig 3 that there was a significant difference ($P < 0.01$) between the ESI values for the conventional SPI and $R_{(0.1)}$. Microfiltering the SPI using the 0.1 μm membrane improved the emulsion-stabilising ability of the resulting retentate. The retentate from the 0.05 μm × 0.02 μm membrane ($R_{0.05}$) did not give rise to a significantly different ESI from that of the SPI. The two permeates $F_{(0.1)}$ and $F_{(0.05)}$ gave significantly lower values of ESI compared with the SPI and the two retentates, but there was no significant difference between the two permeates. The intermediate fraction $FR_{(0.1–0.05)}$ gave an intermediate value of ESI, which was significantly lower than those for the SPI, $R_{(0.1)}$ and $R_{(0.05)}$ but significantly higher than those for $F_{(0.1)}$ and $F_{(0.05)}$.

In the case of the emulsifying activity indices (Fig 4) the retentate $R_{(0.05)}$ gave significantly higher EAI values than those for the SPI and $R_{(0.1)}$, but there was no significant difference in EAI values for the SPI, $R_{(0.1)}$ and $FR_{(0.1–0.05)}$. As with the ESI, the two permeates F(0.1) and F(0.05) resulted in lower EAI values compared with those for the SPI, $R_{(0.1)}$, $R_{(0.05)}$ and $FR_{(0.1–0.05)}$. There was, however, a significant difference in EAI for the two permeates as well, $F_{(0.1)}$ giving a higher value than $F_{(0.05)}$.

From Fig 2 and Table 1 it can be observed that the common feature in the fractions that gave rise to higher ESI and EAI values is the presence of the three high-molecular-weight bands at 65, 76 and 80 kDa. The three fractions $F_{(0.1)}$, $F_{(0.05)}$ and $FR_{(0.1–0.05)}$ that exhibited poor performance in terms of ESI and EAI all lack these high-molecular-weight bands. They were also enriched in lower-molecular-weight bands at 20, 15.6, 14.6 and 3.6 kDa. As noted earlier, the 76 and 80 kDa bands were identified to be the α′ and α subunits of the 7S globulin. The β-7S subunit at 50.1 kDa did not appear to have much influence on the two indices. Another common feature observed for $F_{(0.1)}$, $F_{(0.05)}$ and $FR_{(0.1–0.05)}$ is that they all lacked the 11S acidic subunit at 35 kDa, which showed a strong presence in the retentates. It has been demonstrated

Table 1. Molecular weight profiles with corresponding band percentages for the separated fractions

	Molecular weight bands (kDa)													
Fraction	*80*	*76*	*65*	*60*	*50.1*	*41*	*35*	*33*	*28.4*	*22*	*20*	*15.6*	*14.6*	*3.6*
CE	6	7	6	–	16	–	18	6	4	3	25	–	6	3
SPI	8	11	6	–	16	3	20	6	–	–	23	2	3	2
$R_{(0.1)}$	8	12	6	–	16	3	20	5	–	–	23	2	3	2
$F_{(0.1)}$	–	–	–	9	19	–	–	5	4	4	32	5	17	5
$R_{(0.05)}$	8	10	5	–	15	2	20	5	1	2	26	2	3	1
$F_{(0.05)}$	–	–	–	–	7	–	–	–	–	–	50	26	–	17
$FR_{(0.1–0.05)}$	–	–	–	16	15	–	3	3	–	7	24	16	9	7

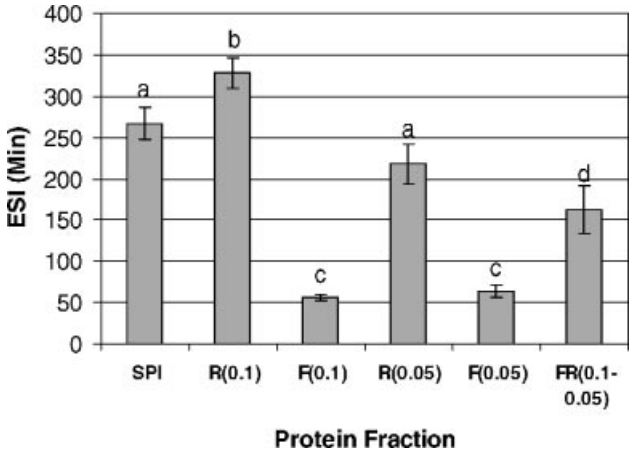

Figure 3. Emulsion stability index (ESI) for the protein fraction-stabilised emulsions: SPI, conventional soy protein isolate; $R_{(0.1)}$, retentate from the 0.1 μm membrane; $F_{(0.1)}$, permeate though the 0.1 μm membrane; $R_{(0.05)}$, retentate from the 0.05 μm × 0.02 μm membrane; $F_{(0.05)}$, permeate through the 0.05 μm × 0.02 μm membrane; $FR_{(0.1–0.05)}$, retentate from the 0.05 μm × 0.02 μm membrane using the 0.1 μm membrane permeate. Protein fractions that do not bear the same letter are significantly different ($P < 0.01$). The error bars represent standard deviations.

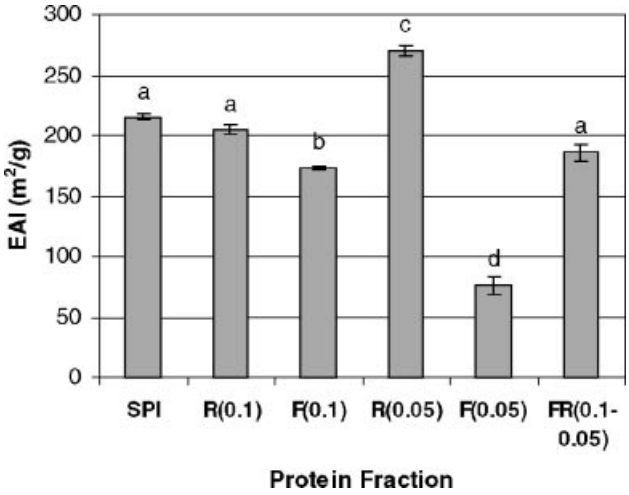

Figure 4. Emulsifying activity index (EAI) for the protein fraction-stabilised emulsions; SPI, conventional soy protein isolate; $R_{(0.1)}$, retentate from the 0.1 μm membrane; $F_{(0.1)}$, permeate though the 0.1 μm membrane; $R_{(0.05)}$, retentate from the 0.05 μm × 0.02 μm membrane; $F_{(0.05)}$, permeate through the 0.05 μm × 0.02 μm membrane; $FR_{(0.1–0.05)}$, retentate from the 0.05 μm × 0.02 μm membrane using the 0.1 μm membrane permeate. Protein fractions that do not bear the same letter are significantly different ($P < 0.01$). The error bars represent standard deviations.

before that 7S-rich SPI fractions have better emulsifying properties than the 11S rich fraction.[24] However, it is also established that the 11S globulin is superior to the 7S globulin in terms of nutritional qualities[25] and gel-forming ability.[26,27]

From the IEF profile of the fractions (Fig 1) it can be seen that the fractions that show a strong presence of molecular species with pI above 5.1 are those that resulted in higher values for ESI and EAI and also gave rise to emulsions with smaller oil droplet size. Fractions richer in species with pI values around 4.6 performed poorly in terms of both ESI and EAI values and also gave rise to relatively large oil droplet sizes.

Oil droplet size

Table 2 shows the oil droplet size (diameter *D*) distribution in the emulsions stabilised by the different fractions, measured 2 h after homogenisation. It was observed that $R_{(0.1)}$-stabilised emulsions gave the smallest average particle size *D*[v,0.5], closely followed by SPI and $R_{(0.05)}$. $F_{(0.05)}$ had the largest particle size, while $F_{(0.1)}$ and $FR_{(0.1–0.05)}$ gave rise to emulsions with particle sizes midway between the relatively small values for SPI, $R_{(0.1)}$ and $R_{(0.05)}$ on the one hand and the comparatively large value for $F_{(0.05)}$ on the other hand. This general observation is quite consistent with the results on ESI and EAI, since emulsions with smaller oil droplet size should be more stable than those with larger droplets. The particle size analyser plots of volume percentage against particle size show $F_{(0.1)}$-, $F_{(0.05)}$- and $FR_{(0.1–0.05)}$-stabilised emulsions resulting in bimodal distributions, suggesting particle agglomeration had taken place by the time of measurement as a result of the instability of the emulsions.

CONCLUSIONS

Microfiltration membranes can be used to enrich high- and low-molecular-weight protein species with different emulsification properties in soy protein isolates. The species giving better emulsion-stabilising ability have been identified as the α′- and α-7S subunits (80 and 76 kDa respectively) of the SPI. It should be noted that while these fractions appear to have higher molecular weight in SDS-PAGE when they are split into subunits, the 11S globulin actually has a higher molecular weight in solution.

Soy protein fractions rich in molecules with iso-

Table 2. Oil droplet size (diameter *D*) distribution in the protein fraction-stabilised emulsions[a]

Protein fraction	D*[v,0.5] (μm)*	D*[3,2] (μm)*	D*[v,0.9] (μm)*	D*[v,0.1] (μm)*	*Span*	*Specific surface area ($m^2\,cm^{-3}$)*
SPI	2.78	2.02	5.50	1.28	1.52	2.9680
$R_{(0.1)}$	1.63	1.35	3.64	0.78	1.75	4.4519
$F_{(0.1)}$	6.60	4.91	11.93	3.26	1.31	1.2224
$R_{(0.05)}$	2.84	2.06	5.65	1.30	1.53	2.9134
$F_{(0.05)}$	13.53	4.88	26.27	1.85	1.80	1.2285
$FR_{(0.1–0.05)}$	6.31	4.67	11.14	3.02	1.29	1.2860

[a] *D*[v,0.5]: 50th percentile by volume; the numbers 0.1 and 0.9 denote the 10th and 90th percentiles respectively. *D*[3,2]: diameter (μm) of a sphere having the same volume as a particle having the average surface area in the distribution. Span: polydispersity of the particles.

electric points above 5.1 exhibited superior functional properties (emulsion stability and emulsifying activity), while those with isoelectric points between 5.1 and 4.5 displayed the poorest functionality. It is therefore recommended that for the production of SPI which is better in the two emulsifying properties, the acidification step should stop at pH 5.1 rather than at the general isoelectric point of 4.5. From an earlier study,[23] the quantity of species with pI between 5.1 and 4.5 is only a small portion (less than 14% w/w) of the total SPI. The loss in protein recovery in this case will thus be minimal.

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