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# A membrane-based process for recovering isoflavones from a waste stream of soy processing <sup>☆</sup>

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## Abstract

Based on a lab-scale aqueous process for making skimmed soymilk, a membrane intensive scheme was developed to recover isoflavones as a separate product from the waste stream while soymilk was prepared as the main product. It was shown that, despite the protein binding, most extracted isoflavones permeated through the selected ultrafiltration membranes. Therefore, instead of solvents or adsorbents, a combination of ultrafiltration and diafiltration was used to separate isoflavones from the proteins and other bean components, and isoflavones were then concentrated by reverse osmosis. A yield of approximately 50% of the total amount of isoflavones in soybeans was eventually obtained in the reverse osmosis retentate, which could be dried to make an isoflavone supplement. With minor modifications, the process was readily adaptable to isoflavone recovery from soy protein processing with defatted soy flour.

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*Keywords:* Soy; Isoflavones; Recovery; Ultrafiltration; Diafiltration; Reverse osmosis; Processing

## 1. Introduction

Isoflavones, due to their potential health benefits, have received increasing attention in the recent years. In addition to the well-established antioxidant effect (Fleury, Welti, Philippoussian, & Magnolato, 1992; Naim, Gestetner, Bondi, & Birk, 1976), these compounds were also found to have weak estrogenic activity (Miksicek, 1993; Molteni, Brizio-Molteni, & Persky, 1995), and as a result, extensive studies show that they are able to fight many diseases, including cardiovascular problems (Carrol, 1991), osteoporosis (Anderson & Carner, 1997), and even certain cancers (Lee et al., 1991; Wei, Wei, Frenkel, Bowen, & Barnes, 1993).

Since soybeans contain far more isoflavones than other legumes and oilseeds, so far almost all isoflavone

products are soy-based. According to processing methods, these can be divided into two types: isoflavone concentrates and isoflavone-enriched soy protein concentrates or isolates. The former ones are typically prepared by extraction with organic solvents, and often in combination with chromatographic techniques (Chang, 2002). Although the products thus made could be of relatively high purity, the use of large quantities of solvents not only poses economical, environmental and safety concerns, but also affects the quality of soy materials by changing their functional properties. The alternative is to make soy protein products containing substantial amounts of isoflavones. As the majority of isoflavones in soybeans is in the form of glucosides, which are water soluble, conventional soy processing tends to incur high losses of isoflavones, and usually only a small fraction (<30%) is recovered in the final product (Wang, Ma, Pagadala, Sherrard, & Krishnan, 1998; Wang & Murphy, 1996). Therefore, in the production of isoflavone-enriched protein isolates, the glucosides must be converted into the more hydrophobic aglycones in order to bind the proteins more firmly.

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Enzymes such as glucosidases or esterases catalyze this type of conversions (Chang, 2002). Enzyme treatment increased the isoflavone recovery (up to 80%), but adds to the processing cost. Moreover, deactivation of enzyme by heat may also alter the functionalities of soy protein isolates.

The isoflavone-enriched protein isolates may be taken directly as isoflavone supplements. Most soy protein products manufactured, however, are currently intended for use as ingredients in various complex food systems, where isoflavones not only would be much diluted but even undesired since they are responsible for the bitter flavour of soy products (Okubo et al., 1992). Therefore, it is desirable to recover isoflavones as a separate product while making high-quality soy protein products. In this study, a new approach using aqueous processing for recovering isoflavones is reported. It actually utilizes the waste stream from a previously reported process to make skimmed soy milk (Xu, Kumar, & Lamb, 2004), which employs a high water-to-soybean ratio to facilitate skimming by centrifugation, and the resultant dilute soymilk is then concentrated by ultrafiltration (UF), generating a final liquid qualified as skimmed soymilk, and a permeate as the major waste stream of the process. As the permeate contains significant amounts of isoflavones, it would be environmentally and economically beneficial to treat in order to recover these high-value compounds. Membrane processing is the key technique for treatment to achieve the objective of this study. Although the process was developed to recover isoflavones from full-fat beans, it could be readily adapted to soy protein processing from defatted flour.

## 2. Materials and methods

### 2.1. Preparation of skimmed soymilk

The procedure of described by Xu et al. (2004) was followed. Dry soybeans were purchased from a local grocery. For each run, 100 g beans presoaked in water overnight were ground at a water-to-dry bean ratio of 5:1. It was diluted to a ratio of 30:1 and extracted for 30 min with continuous stirring at room temperature. After straining, the dilute milk was passed through an Armfield disc bowl centrifuge FT-15 (Armfield Limited, Hampshire, England) at 11,000 rpm. In some runs the milk was boiled for 15 min before centrifugation. The defatted, dilute milk was then concentrated 3.5–4 times by UF in a high output stirred cell (Millipore Ltd., Etobicoke, Ontario) using a regenerated cellulose membrane with a molecular weight cut-off (MWCO) of 30,000 (Millipore Ltd., Bedford, MA). A *trans*-membrane pressure of ~350 kPa was maintained by compressed N<sub>2</sub> gas. Along with soymilk, a permeate stream was produced in ultrafiltration process. UF was done at

either at room temperature or 50 °C. In the latter case, the entire stirred cell was immersed in a Polyscience heated/refrigerated circulating water bath Model 1157 (VWR International Ltd., Ville Mont-Royal, QC).

When defatted soy flour was used as the starting material, the process was modified by skipping certain steps such as blending, straining and defatting, and the spent flour residue was separated using an IEC CR-6000 swing basket centrifuge (International Equipment Co., Needham, MA). Thus, a protein solution was produced in the end instead of soymilk.

### 2.2. Diafiltration of skimmed soymilk

In this set of experiments, the preparation of skimmed soymilk was scaled up by combining five batches of boiled and defatted dilute milk before UF, each batch prepared with 100 g beans, and UF was conducted at 50 °C in a Millipore PLTK Prep/Scale TFF cartridge (Millipore Ltd., Bedford, MA) using a regenerated cellulose membrane with a MWCO of 30 kDa. The retentate was circulated at a flow rate of 3.5 l/min by a micropump Model 646 (Micropump Corp., Concord, CA). The dilute soymilk was concentrated from about 10 to 3.2 l. The concentrated soymilk was then continuously diafiltered with 10 l water using the same system. The temperature of the retentate was maintained at 50 °C on a Mirak digital stirrer SP72725 (Barnstead International, Dubuque, IA). Samples were taken from the pooled permeate of diafiltration (DF) at intervals of 2 l for isoflavone analysis.

### 2.3. Ultrafiltration of permeate

The permeate of DF was combined with that of UF. The pooled permeate was ultrafiltered in a PLAC Prep/Scale TFF Cartridge (Millipore Ltd., Bedford, MA) using a much tighter regenerated cellulose membrane with a MWCO of 1 kDa. Hence, a higher *trans*-membrane pressure of ~550 kPa was needed. The same hot plate stirrer as above was used to maintain the temperature of the retentate at 50 °C. This UF step continued until the feed volume was reduced by about 90%.

### 2.4. Recovery of isoflavones from permeate by reverse osmosis

The permeate of UF using the above 1 k membrane was concentrated 10–15 times by reverse osmosis (RO) at room temperature, using a plate-and-frame filtration system (three sheets 35 × 10 cm). A thin-film RO membrane, Desal-3 with a 98% salt rejection was used (GE Osmonics, Vista, CA). During the RO process a pressure drop of 1400 kPa and a feed recirculation rate of 3.2 l/min were maintained.

## 2.5. Analyses

For the determination of isoflavones in all the samples, the method of Klump, Allred, MacDonald, and Ballam (2001) was followed using a Hewlett Packard Series 1100 HPLC system with a Zorbax SB-C18 reversed-phase column (250 × 4.6 mm i.d.) (Agilent Technologies, Wilmington, DE). In this method, esterified isoflavones were converted to glucosides by alkaline hydrolysis, thus only two types of isoflavones were of concern in this study, aglycones including daidzein, genistein and glycitein, and glucosides including daidzin, genistin and glycitin. The standards of all six compounds were purchased from Indofine Chemical Co., Somerville, NJ. Since these compounds have different molecular weights, for meaningful comparison, the isoflavone content was expressed as  $\mu\text{mol/g}$  sample.

Other analyses included nitrogen determination using the Kjeldahl method, with a Büchi K-424 digester and a Büchi K-314 distillation unit (Brinkmann Instruments, Mississauga, Ontario), and total phosphorus determination by inductively coupled plasma atomic emission spectrometry (ICP-AES) with a Perkin–Elmer Optima 3000 (Perkin–Elmer Instruments, Shelton, CT).

## 3. Results and discussion

### 3.1. Yield of isoflavones in 30 k UF permeate

The lab-scale process to make skimmed soymilk (Xu et al., 2004) yielded four product streams: a concentrated skimmed soymilk, a spent bean residue, a fat-enriched “cream” phase and a UF permeate. The total amount of isoflavones in soybeans was divided up among these streams. The results of isoflavone analyses were all mean values of duplicates unless otherwise indicated. The average coefficient of variation in each run was calculated to be less than 5%, indicating small standard deviations for all samples. During the extraction with a high water ratio of 30:1, about 90% of isoflavones present in the beans ended up in the dilute soymilk (Table 1). After centrifugal defatting, the sub-

sequent UF step split the extracted isoflavones almost evenly between the concentrated milk and the permeate at approximately 30% of the total amount, indicating the binding of isoflavones to soy proteins. Otherwise, at a concentration factor of 3.5–4, a membrane with such a high MWCO (30 k) would have permeated much more isoflavones as their molecular weights (MW) are in the range from 250 to 550. It was noted that, although isoflavone glucosides were predominant over aglycones in the beans, total glucosides in all product streams combined were reduced by half while that of the aglycones increased by more than five times. In the concentrated soymilk as well as spent bean residue, the aglycones became the major type of isoflavones. This change in amount was apparently a result of the enzymatic hydrolysis by a native enzyme  $\beta$ -glucosidase in soybeans (Ha, Morr, & Seo, 1992), which converted much of the glucosides to the aglycones during a processing period of more than 20 h. Since isoflavone aglycones, as mentioned earlier, tend to have a stronger protein-binding ability than the glucosides, due to their higher hydrophobicity, it may explain the observation that nearly a third of total isoflavones was retained by the 30 k UF membrane in the concentrated skimmed soymilk, resulting in a low yield of isoflavones in the permeate. About 17% of the total isoflavone amount could not be accounted for, likely a result of line “losses” and analytical errors.

When the dilute soymilk was cooked (boiled) to enhance the effect of centrifugal defatting, the enzyme was coincidentally deactivated, hence a lower conversion rate of glucosides to aglycones (Table 2). There was, however, still about 30% of the total isoflavone amount remaining in the soymilk likely through binding to the proteins. So the yield in the permeate only increased slightly, suggesting that the binding by the glucosides was also considerable. To weaken the binding, the temperature of the low-fat dilute soymilk was elevated to 50 °C, the maximum recommended operating temperature for the UF membrane, and was maintained throughout the entire UF step. As a result isoflavone yield increased to more than 45% in the permeate (Fig. 1) while that of the skimmed soymilk dropped to

Table 1  
Isoflavone distribution of soymilk processing without cooking

| Stream               | Mass (g)          | Aglycone content ( $\mu\text{mol/g}$ ) | Aglycone amount ( $\mu\text{mol}$ ) | Glucoside content ( $\mu\text{mol/g}$ ) | Glucoside amount ( $\mu\text{mol}$ ) | Total amount ( $\mu\text{mol}$ ) | Percentage (%) |
|----------------------|-------------------|--|-------------------------------------|---|--------------------------------------|----------------------------------|----------------|
| Soybeans             | 100               | 0.350                                  | 35.0                                | 4.65                                    | 465                                  | 500                              | 100            |
| Spent bean residue   | 28.3              | 1.45                                   | 41.0                                | 0.580                                   | 16.4                                 | 57.4                             | 11.5           |
| Concentrated soymilk | 610               | 0.214                                  | 130                                 | 0.0342                                  | 20.9                                 | 151                              | 30.3           |
| UF Permeate (30 k)   | 1922              | 0.0278                                 | 53.4                                | 0.0616                                  | 118                                  | 172                              | 34.4           |
| “Cream”              | 194               | 0.0915                                 | 17.8                                | 0.0805                                  | 15.6                                 | 33.4                             | 6.7            |
| Change <sup>a</sup>  | n.a. <sup>b</sup> | n.a.                                   | 208                                 | n.a.                                    | -294                                 | -86                              | -17.2          |

<sup>a</sup> All product streams minus starting material (soybeans).

<sup>b</sup> Not applicable.

Table 2  
Isoflavone distribution of soymilk processing with cooking

| Stream               | Mass (g)          | Aglycone content ( $\mu\text{mol/g}$ ) | Aglycone amount ( $\mu\text{mol}$ ) | Glucoside content ( $\mu\text{mol/g}$ ) | Glucoside amount ( $\mu\text{mol}$ ) | Total amount ( $\mu\text{mol}$ ) | Percentage (%) |
|----------------------|-------------------|--|-------------------------------------|---|--------------------------------------|----------------------------------|----------------|
| Soybeans             | 100               | 0.350                                  | 35.0                                | 4.65                                    | 465                                  | 500                              | 100            |
| Spent bean residue   | 27.0              | 1.30                                   | 35.1                                | 0.610                                   | 16.5                                 | 51.6                             | 10.3           |
| Concentrated soymilk | 536               | 0.102                                  | 54.6                                | 0.177                                   | 94.8                                 | 150                              | 30.0           |
| UF Permeate (30 k)   | 1927              | 0.0105                                 | 20.2                                | 0.0906                                  | 175                                  | 195                              | 39.0           |
| “Cream”              | 154               | 0.0540                                 | 8.3                                 | 0.123                                   | 18.9                                 | 27.3                             | 5.5            |
| Change <sup>a</sup>  | n.a. <sup>b</sup> | n.a.                                   | 83.2                                | n.a.                                    | -160                                 | -76.1                            | -15.3          |

<sup>a</sup> All product streams minus starting material (soybeans).

<sup>b</sup> Not applicable.

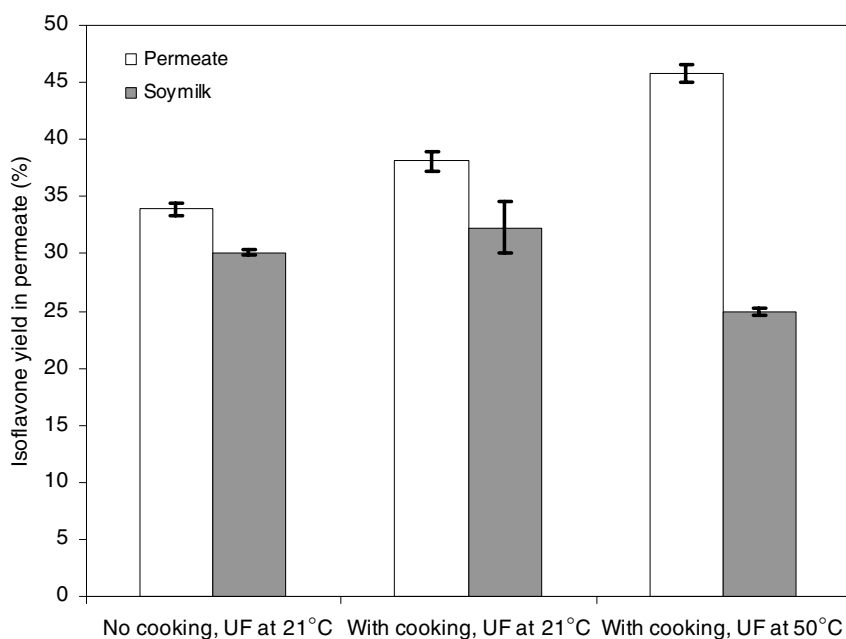


Fig. 1. Yield of isoflavones in UF permeate as % of the total amount in soybeans. The results are presented as the total amount of isoflavones in soybeans. The error bars are based on duplicate runs.

25%. Another obvious effect of elevating the temperature was significant increase in UF permeate flux from approximately 15 to over 45 l/m<sup>2</sup> h, as the higher temperature decreased viscosity and increased diffusivity, hence an increased mass transfer coefficient and a higher flux.

### 3.2. Yield of isoflavones by diafiltration of soymilk

It has now been established that at the current concentration factor, UF alone, even at an elevated temperature could only transfer less than 50% of the total isoflavones in the permeate. Although further concentration of the milk may increase the yield in the permeate, it was uneconomical, and possibly detrimental to the membrane as the protein concentration of soymilk was already greater than 3% at this point. Therefore, a sensible choice was continuous DF of soymilk to im-

prove the isoflavone yield in the permeate. In DF, water is added at the same rate as the permeate is removed, so the volume of retentate is maintained constant. It is described by the following equation (Cheryan, 1986):

$$\frac{C_f}{C_0} = e^{\frac{V_W}{V_R}(R-1)} = e^{D_V(R-1)}, \quad (1)$$

where  $C_0$  is the initial solute concentration,  $C_f$  the final solute concentration,  $V_W$  the volume of water added,  $V_R$  the volume of retentate (concentrated soymilk in this case),  $D_V$  the diavolume,  $\frac{V_W}{V_R}$ , and  $R$  is the rejection coefficient of solute. In reality, only the solute concentration in the pooled DF permeate  $C_P$  is of concern, and it can be calculated as follows:

$$C_P = \frac{C_0[1 - e^{D_V(R-1)}]}{D_V}. \quad (2)$$

In this study, the solutes of concern are various isoflavones in soymilk, and they were grouped into two

types, glucosides and aglycones, to simplify calculations. Since the MWCO of the membrane used in this case, 30 k, was far greater than the MWs of all isoflavones, ideally they would all permeate through the membrane freely, i.e.,  $R$  was zero for all isoflavones. It has, however, been shown earlier that isoflavone–soy protein binding is considerable, therefore the values of actual  $R$  varied between 0 and 1. The characteristic equation of UF (Cheryan, 1986) could be used for the estimation of  $R$  in this case:

$$\frac{C_f}{C_0} = \left( \frac{V_0}{V_f} \right)^R = (CF)^R, \quad (3)$$

where  $C_0$  is the isoflavone concentration in dilute soymilk,  $C_f$  the isoflavone concentration in concentrated soymilk,  $V_0$  the initial volume of feed,  $V_f$  the final volume of retentate, and  $CF$  is the concentration factor,  $\frac{V_0}{V_f}$ . The overall  $R$ -values of glucosides and aglycones were thus estimated to be 0.150 and 0.517, respectively. It can be seen that their difference in protein binding was reflected upon their diverse  $R$ -values. As the aglycones were more hydrophobic than the glucosides, they tended to bind to soy proteins more firmly, thus were more likely to be retained by the membrane, hence a higher  $R$ -value.

Based on the  $R$ -values, the isoflavone concentrations in the pooled DF permeate  $C_p$  were predicted using Eq. (2) and plotted against the diafiltration volume in Fig. 2, and they were compared to the actual concentrations determined experimentally. While descending

$C_p$  curves were observed for the glucosides as the DF process continued, relatively flat curves were seen for aglycones. As can be seen, for the glucosides the experimental values of  $C_p$  matched the calculated ones so closely that the two curves almost overlapped. The experimental  $C_p$  values, however, deviated from the calculated set in the case of aglycones, particularly during the early stage of the DF process ( $D_v < 3$ ). The discrepancies were likely a result of firm protein binding by the aglycones, which may vary considerably with the aglycones concentration, thus changing the overall permeability of these compounds through the membrane. Since the glucosides amounts were overwhelming in the total, it was valid to use the above formulas in determining the volume of water necessary to achieve a certain yield of isoflavones in the permeate. Although a larger water volume would result in a higher isoflavone yield, too much water from DF would require additional processing, resulting in higher costs.

In this study, it was decided to end the DF step when the diavolume  $D_v$  reached 3.1, which is equivalent to a water volume of 10 l on a scale of 500 g soybeans for starting material. At a similar permeate flux to that of the stirred cell, the membrane cartridge with a membrane area of 0.56 m<sup>2</sup> was able to deliver a permeate flow rate of 25 l/h, thus speeding up the process significantly. Isoflavone balance among all streams of the processing with DF (Table 3) showed a yield as high as 75% in the combined permeate of UF and DF, which

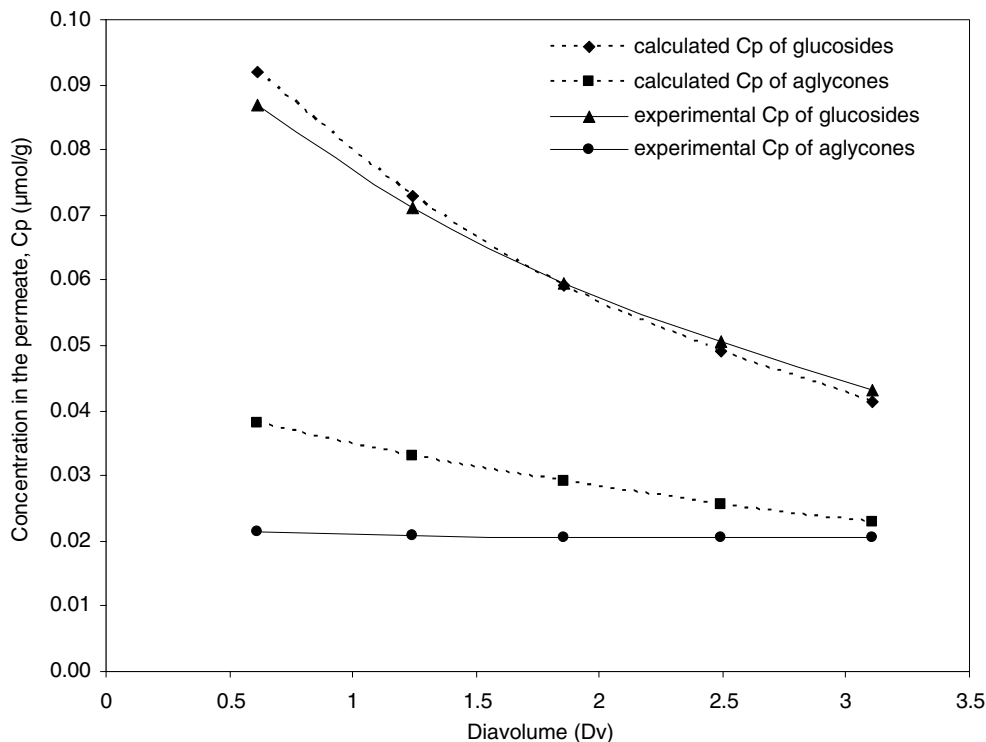


Fig. 2. Comparison of calculated and experimental isoflavone concentrations in the permeate of diafiltration.

Table 3  
Isoflavone distribution of soymilk processing with DF

| Stream                         | Mass (g)          | Aglycone content ( $\mu\text{mol/g}$ ) | Aglycone amount ( $\mu\text{mol}$ ) | Glucoside content ( $\mu\text{mol/g}$ ) | Glucoside amount ( $\mu\text{mol}$ ) | Total amount ( $\mu\text{mol}$ ) | Percentage (%) |
|--------------------------------|-------------------|--|-------------------------------------|---|--------------------------------------|----------------------------------|----------------|
| Soybeans                       | 500               | 0.350                                  | 175                                 | 4.65                                    | 2325                                 | 2500                             | 100            |
| Spent bean residue             | 131               | 1.34                                   | 176                                 | 0.318                                   | 41.6                                 | 218                              | 8.7            |
| Concentrated soymilk           | 3219              | 0.0512                                 | 165                                 | 0.0185                                  | 59.6                                 | 225                              | 9.0            |
| “Cream”                        | 498               | 0.0685                                 | 34.1                                | 0.114                                   | 56.7                                 | 90.8                             | 3.6            |
| Pooled UF & DF permeate (30 k) | 20017             | 0.0217                                 | 435                                 | 0.0719                                  | 1439                                 | 1874                             | 75.0           |
| Change <sup>a</sup>            | n.a. <sup>b</sup> | n.a.                                   | 635                                 | n.a.                                    | -728                                 | -92.2                            | -3.7           |

<sup>a</sup> All product streams minus starting material (soybeans).

<sup>b</sup> Not applicable.

was equivalent to an increase by more than 50% over the results using UF alone. Another notable change associated with DF was that the unaccountable fraction was reduced to less than 4%. This was possibly due to more accurate isoflavone analyses of the permeate than those of soymilk whose isoflavone concentrations tended to be underestimated because of the isoflavone–protein binding. Therefore, with a greater amount of isoflavones in the permeate, a better balance could be expected between the starting material and all product streams, represented by a smaller unaccountable fraction.

### 3.3. UF of permeate with 1 k membrane

In soymilk processing, a relatively open membrane with a MWCO of 30 k for both UF and DF was used. It was able to retain large-molecular-weight solutes such as soy proteins, but allowed components with low molecular weights to pass, and besides isoflavones, they may include peptides, free amino acids, saccharides and others. Among these, phytates could be a concern as they were considered as anti-nutrient for their ability to bind minerals. Soybeans typically contain 2–3% phytates, accounting for up to 80% of the total phosphorus, and with the chelated metal ions they have molecular weights above 1000. Therefore, to reduce the amount of these impurities with respect to isoflavones, another UF step was introduced using a much tighter membrane with a MWCO of 1 k, to treat the permeate of UF and DF with the 30 k membrane. The low MWCO signifi-

cantly slowed down the flux of the permeate to about 10 l/m<sup>2</sup> h, as compared to 45 l/m<sup>2</sup> h in UF with the 30 k membrane, although both cartridges had the same membrane area of 0.56 m<sup>2</sup>. The results in Table 4 showed that, while removing most of the undesired solutes represented by non-protein nitrogen and total phosphorus, the 1 k membrane let both types of isoflavones pass quite easily. The rejection coefficients of glucosides and aglycones for the membrane were estimated to be 0.224 and 0.051 for glucosides and aglycones, respectively. Thus, almost 80% of the amount of isoflavones in the first permeate (30 k) was recovered in the second permeate (1 k), equivalent to some 60% of the total amount in soybeans, while about 10% remained in the 1 k retentate.

### 3.4. Recovery of isoflavones by RO

The above permeate of UF with the 1 k membrane was treated by RO to concentrate the isoflavones at an average permeate flux around 24 l/m<sup>2</sup> h. The mass balance shown in Table 5 was for this step alone, thus the permeate from 1 k membrane being the “starting” material. More than 75% of the amount of isoflavones in the 1 k permeate was recovered in the RO retentate, equivalent to a recovery of about 46% based on the total amount in soybeans. Only a small fraction (~5%) ended up in the RO permeate. The retentate trapped in the pipelines of the RO system was largely responsible for a mass loss of close to 600 g, resulting in an unaccountable

Table 4  
UF of permeate using 1k membrane<sup>a</sup>

| Stream        | Isoflavone glucosides ( $\mu\text{mol/g}$ ) | Isoflavone aglycones <sup>b</sup> ( $\mu\text{mol/g}$ ) | NPN <sup>c</sup> (%) | Total P <sup>d</sup> (ppm) |
|---------------|---|---|----------------------|----------------------------|
| 30 k Permeate | 0.0725                                      | 0.0225 <sup>AC</sup>                                    | 0.037                | 75.6                       |
| 1 k Permeate  | 0.0680                                      | 0.0168 <sup>AB</sup>                                    | n.d. <sup>e</sup>    | 23.9                       |
| 1 k Retentate | 0.128                                       | 0.0256 <sup>C</sup>                                     | 0.50                 | 572                        |

<sup>a</sup> All values are means of triplicates. All values in a column are significantly different from one another unless otherwise indicated ( $p < 0.05$ ).

<sup>b</sup> Values not sharing a common letter in this column are significantly different from each other ( $p < 0.05$ ).

<sup>c</sup> Non-protein nitrogen, nitrogen  $\times$  6.25.

<sup>d</sup> Total phosphorus.

<sup>e</sup> Not detected.

Table 5  
Recovery of isoflavones from soymilk processing by reverse osmosis (RO)

| Stream                           | Mass (g) | Aglycone content ( $\mu\text{mol/g}$ ) | Aglycone amount ( $\mu\text{mol}$ ) | Glucoside content ( $\mu\text{mol/g}$ ) | Glucoside amount ( $\mu\text{mol}$ ) | Total amount ( $\mu\text{mol}$ ) | Percentage <sup>a</sup> (%) |
|----------------------------------|----------|--|-------------------------------------|---|--------------------------------------|----------------------------------|-----------------------------|
| Permeate of UF with 1 k membrane | 18104    | 0.0165                                 | 299                                 | 0.0673                                  | 1218                                 | 1517                             | 60.7                        |
| Permeate of RO                   | 15786    | 0.00508                                | 80.2                                | 0.00331                                 | 52.2                                 | 132                              | 5.3                         |
| Retentate of RO                  | 1730     | 0.101                                  | 174                                 | 0.570                                   | 986                                  | 1160                             | 46.4                        |
| “Loss” <sup>b</sup>              | -588     | n.a. <sup>c</sup>                      | -44.8                               | n.a.                                    | -180                                 | 225                              | -8.9                        |

<sup>a</sup> As of total amount of isoflavones in soybeans.

<sup>b</sup> Unaccountable by RO.

<sup>c</sup> Not applicable.

Table 6  
Isoflavone distribution of processing with defatted soy flour

| Stream                            | Mass (g)          | Aglycone content ( $\mu\text{mol/g}$ ) | Aglycone amount ( $\mu\text{mol}$ ) | Glucoside content ( $\mu\text{mol/g}$ ) | Glucoside amount ( $\mu\text{mol}$ ) | Total amount ( $\mu\text{mol}$ ) | Percentage (%) |
|-----------------------------------|-------------------|--|-------------------------------------|---|--------------------------------------|----------------------------------|----------------|
| Soy flour                         | 500               | 0.244                                  | 122                                 | 8.61                                    | 4304                                 | 4426                             | 100            |
| Spent flour residue               | 248               | 1.76                                   | 436                                 | 1.74                                    | 432                                  | 868                              | 19.6           |
| Concentrated soy protein solution | 3100              | 0.0212                                 | 65.8                                | 0.0185                                  | 59.6                                 | 126                              | 2.9            |
| Retentate of UF with 1 k membrane | 1996              | 0.0206                                 | 41.0                                | 0.230                                   | 460                                  | 501                              | 11.3           |
| Permeate of RO                    | 16167             | 0.00601                                | 97.0                                | 0.00554                                 | 89.5                                 | 187                              | 4.2            |
| Retentate of RO                   | 1490              | 0.126                                  | 188                                 | 1.28                                    | 1905                                 | 2093                             | 47.3           |
| Change <sup>a</sup>               | n.a. <sup>b</sup> | n.a.                                   | 706                                 | n.a.                                    | -1358                                | -651                             | -14.7          |

<sup>a</sup> All product streams minus starting material (soybeans).

<sup>b</sup> Not applicable.

amount of 9% of the total isoflavones. This “loss” plus those in the upstream steps made up a total isoflavone imbalance of approximately 15%. An aliquot was taken from the RO retentate and oven dried at 85 °C overnight to yield a solid with an isoflavone content of 27.1  $\mu\text{mol/g}$  (10.9 mg/g), based on which it was estimated that a total of 42 g of such a solid could be produced from 500 g soybeans representing a mass yield of 8.4%.

This process could also be adapted to soy protein production using defatted soy flour as the starting material (Table 6), where a final isoflavone recovery of 47.3% was obtained, similar to that of soymilk processing. As the defatted flour was likely toasted to result in a lower solubility, a higher amount of spent residue, almost half the amount of soy flour, was produced than that in the case of soymilk (about 30%), thus allowing more isoflavones to remain in the residue. The concentrated protein solution however accounted for a lower percentage of the total amount of isoflavones than the soymilk, and the total line “losses” were close to those of soymilk processing (about 15%). A representative sample of the retentate of RO in this case was also oven dried as above, and this dried solid had an isoflavone content of 37.1  $\mu\text{mol/g}$  (15.2 mg/g), which may be used as an isoflavone supplement. Based on the content it was again estimated that about 56 g of such a product could be made from 500 g of soy flour. Since the permeate was already treated by RO, it was probably clean enough to

reuse without any further treatment, thus making it essentially a “zero discharge” process.

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