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Ultra- and Nano-Filtration Process Optimization of Isoflavones and Oligosaccharides from Sunmul

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Abstract Optimal conditions of ultrafiltration (UF) and nanofiltration (NF) were investigated for separation and concentration of isoflavones and oligosaccharides from Sunmul. Levels of COD, BOD, and suspended solids (SS) in UF and NF permeates were also determined to evaluate effectiveness of these processes for reducing water pollution. Optimal UF operation conditions to achieve minimal fouling and maximal flux were 33-34°C operating temperature and 2.3-2.4 bar trans-membrane pressure. Recovery yields of isoflavones and oligosaccharides in UF retentate were 11.49-28.16% and 12.77-27.57%, respectively. Increase in volumetric concentration factor (VCF) resulted in more functional compounds of isoflavones and oligosaccharides passing through UF membrane. Total isoflavone and oligosaccharide yields decreased by 3% as VCF increased from 6.0 to 8.0 and from 8.0 to 10.0, while decreased significantly by 10% as VCF decreased from 4.0 to 6.0. Optimal NF operating conditions were 192-195 psig operating pressure at 30-33°C. Total yields of isoflavones and oligosaccharides significantly decreased at VCF 8.0, whereas did not decrease up to VCF 6.0 during NF operation. Therefore, VCF 6.0 was recommended for economical process. COD and BOD decreased by more than 98% after NF process, and SS were not detected after UF process. These results indicated sequential filtration process was useful for separation of isoflavones and oligosaccharides from Sunmul and for reducing water contaminants.

Keywords: Sunmul, isoflavones, oligosaccharides, ultrafiltration, nanofiltration, response surface methodology

Introduction

Sunmul, the tofu whey produced after pressing protein coagulants during tofu formation, has been regarded as serious water contaminant in tofu industry. The solid content in Sunmul is 2-3% and contains mainly small molecular weight materials such as low molecular protein, peptides, lipids, oligosaccharides, and some functional compounds (1, 2). Among the functional food materials, isoflavones are one of the most interesting natural compounds. Genistein and genistin, among the twelve identifiable isoflavones, have been studied for their prevention and treatment of a number of chronic diseases, including cancer, coronary heart disease (CHD), osteoporosis, and diabetes (3, 4). Their major anticarcinogenic effects were studied on breast cancer, large intestine cancer, and prostatic carcinoma (5). Through numerous attempts on industrial separation of isoflavone, several isoflavone products have been successfully marketed.

Non-digestible oligosaccharides have relatively low calories, with almost no increase in the blood sugar and serum cholesterol levels. They reach large intestine intact, without acidolysis or hydrolysis in the intestinal tract, and preferentially promote the growth of endogenous bacteria such as *bifidobacteria* and *lactobacilli*, thereby promoting the host health (6-8). Other functional materials from Sunmul were studied as well. These compounds included oligosaccharides, such as raffinose and stachyose, functional peptides, saponin, phytate, and pinitol (9, 10).

Membrane separation techniques using microfiltration (MF), ultrafiltration (UF), and nanofiltration (NF) have been applied to the separation and concentration of useful compounds. The molecular weight cut off (MWCO) of NF process is down to 180, and NF can be differentiated from reverse osmosis (RO) through the pressure-driven separation. Removal of relatively large molecular weight compounds such as tofu debris and foaming compounds is prerequisite to increasing the NF efficiency, because the fouling rate on the membrane is inversely proportional to the permeate flux (11, 12). Several works have been reported on the application of NF in food industry, i.e. the concentration and recovery of aromas from the fruit juice, purification of thin juice, concentration of sugar, and demineralization of milk (13, 14).

In the present study, optimal pressure and temperature conditions were investigated for UF and NF of Sunmul to minimize the fouling effect and maximize the permeate flux, and the recovery yields of isoflavones and oligosaccharides were determined. Additionally, the effects of filtration on food waste control were studied by measuring COD, BOD, and SS of the filtration permeates.

Materials and Methods

Materials Sunmul (soybean curd whey, tofu whey) was obtained from Doosol Corporation (Yeisan-Gun, Korea). Membrane filters were purchased from Millipore Corporation (Bedford, MA, USA). Nominal pore diameter of depth-type MF membrane used for pre-filtration was 5 mm. Nominal molecular weight cut-off (MWCO) and membrane surface area of cassette-type UF membrane were 10,000 daltons and 0.1 m^2, respectively. Nominal salt rejection and membrane surface area of spiral-type NF membrane were 65% and 0.4 m^2, respectively. Isoflavone standards (daidzein, daidzin, genistein, genistin, glycitein, and glycitin) and oligosaccharides standards (sucrose,

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raffinose, and stachyose) were purchased from Sigma Chemical Corporation (St. Louise, MO, USA).

Membrane process and concentration Experiments for membrane separation and concentration were performed in a batch mode using a pilot-scale plant (Fig. 1). MF was carried out to remove large molecules such as insoluble substances and suspended solids (SS) from Sunmul, followed by UF for selection of Volumetric concentration factor (VCF) of the retentate. UF permeate was then further separated by NF with recycling of the retentate to concentrate the isoflavones and oligosaccharides. The entire membrane process was repeated three times. UF and NF retentates were freeze-dried for the analysis of isoflavones and oligosaccharides. UF and NF permeates were used to measure COD, BOD, and SS levels.

Experimental design for RSM by UF and NF system Optimization of UF and NF filtrations to separate and concentrate Sunmul was carried out using response surface methodology (RSM) (15). The selected experimental design, so called the central composite design, had two factors and three levels (Table 1), with two replicates in the central point. The permeate flux is affected by two independent variables, the operating temperature (OT) and the trans membrane pressure (TMP) of the system. Data were analyzed using the RSREG, PLOT, and REG procedures of SAS (SAS Institute Inc., Cary, NC, USA) and were fitted to the second-order polynomial equation to optimize the conditions of the membrane process. The results of data analysis are presented as response surface plots and contour maps. The general second-order equation is expressed as follows: $Y = \beta_1 + \beta_1 X_i + \beta_2 X_j + \beta_{12} X_i X_j + \beta_{11} X_i^2 + \beta_{22} X_j^2$, where Y is the dependent variable, β is the regression coefficient in the model equation, and X is the independent variable.

Average flux Average flux was calculated as follows:

$$\text{Average flux} = \frac{\text{mL permeate}}{\text{Unit area of membrane (m}^2\text{)} \times \text{Operating time (hr)}}$$

Volumetric concentration factor (VCF)
VCF was calculated as follows:

$$VCF = \frac{V_{initial}}{V_{final}}$$

where $V_{initial}$ is the volume (mL) of the sample before the membrane process, and V_{final} is the volume (mL) of the retentate during the membrane process.

Fouling index (b) Several mathematical models of the fouling process available in the literature were evaluated. Most models are empirical and generally of the exponential or power law form (16). Our data best fit the power law form as shown below:

$J_v = J_o t^{-b}$

where J_v is the permeate flux after t min, J_o is the permeate flux after 1 min, t is the time of operation, and b is the fouling index.

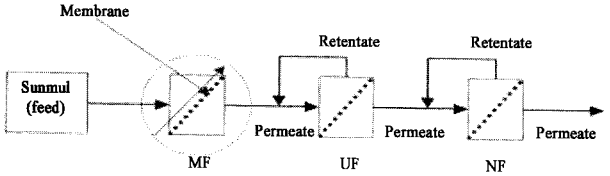

Fig. 1. Flow diagram of Sunmul separation process by UF- and NF-filtrations.

Table 1. Coded levels for independent variables used in experimental design for UF and NF systems

X_i	Flux conditions	Levels		
		-1	0	+1
	UF system			
X_1	Temperature (°C)	10	25	40
X_2	TMP (bar)	1.5	2.0	2.5
	NF system			
X_1	Temperature (°C)	10	25	40
X_2	Pressure (psig)	150	180	210

Analysis of COD, BOD, and SS The levels of COD (chemical oxygen demand) and BOD (biological oxygen demand) were measured by the official methods (17). A gravimetric method was used to determine the SS level by weighing the residual amount of dried solids on the glass fiber filter.

HPLC analysis of isoflavone Retentates from UF and NP filtration process were freeze-dried and used for isoflavone analysis. One gram of freeze-dried sample was extracted with 20 mL of 80% ethanol for 1 hr using an ultrasonicator. The extract was centrifuged at 10,000×*g* for 20 min, and the supernatant was filtered through a syringe filter (0.22 mm, Waters Co., Milford, MA, USA). Analysis of isoflavone was performed by HPLC (Waters Co.) using a Waters 486 absorbance UV detector at 254 nm and an XTerra™ RP_{18} column (5 mm, 4.6 × 250 mm, Waters Co.). The mobile phases for HPLC consisted of solvent (A), 0.1% (v/v) acetic acid in water, and (B), 0.1% (v/v) acetic acid in acetonitrile. The solvent gradient was as follows: the fraction of solvent B increased linearly from 15 to 23% over initial 40-min period, followed by 27% increase up to 30 min, to 35% up to the following 15 min, and finally to 40% within last 5 min at a flow rate of 1 mL/min. Standard curve was obtained by plotting the standard mixture solution as a function of the peak area in HPLC chromatograms. The amount of each isoflavone isoform in the sample was quantified based on the standard curves generated by calculating the HPLC peak areas of highly pure commercial daidzein, daidzin, genistein, genistin, glycitein, and glycitin, and averaged from results of triplicate analyses.

HPLC analysis of oligosaccharide The amount of each oligosaccharide was determined following the procedure of Mok *et al.* with some modifications (18). One gram of freeze-dried samples was extracted with 20 mL of 10% ethanol for 1 hr using an ultrasonicator. The extract was centrifuged at 10,000 × *g* for 20 min. The supernatant (0.5 mL) was added to 0.5 mL of 10% lead acetate and 0.5 mL

water, and centrifuged at 10,000 × *g* for 5 min to remove proteins. The supernatant was added 10% oxalic acid to precipitate surplus lead acetate, followed by filtration through syringe filter (0.22 μm, Waters Co.) prior to HPLC analysis. Oligosaccharides were analyzed using an HPLC system (Waters Co.) equipped with a Water 2414 Refractive index detector and a Carbohydrate analysis column (3.9 × 300 mm). The mobile phase of 65% acetonitrile was used at the flow rate of 1.3 mL/min for 20 min. Standard curves were prepared for sucrose, raffinose, and stachyose. The amount of each component was calculated as described in the isoflavone analysis section.

Results and Discussion

Optimum condition of ultafiltration The response surface for optimal UF operating conditions is shown in Fig. 2. Exploration of response surface indicated a complex interaction between the independent variables such as TMP and OT. The levels of TMP for UF process and OT were 1.5, 2.0, and 2.5 bar, and 10, 25, and 40°C, respectively. Monitoring of TMP, the average applied pressure on the UF membrane from the feed side to the filtrate side, is important to optimize the filtration process by achieving the highest flux without generating excessive pressure. RSREG analysis revealed the second-order polynomial equation was best fitted to the experimental data. The regression model equation for the permeate flux was expressed as follows: Flux = -32.555556 + 50.452381(OT) + 0.233333(OT)(TMP) - 10.571429$(OT)^2$ + 0.008254 $(TMP)^2$ (R^2=0.9850). The fitted polynomial equation was shown as a three-dimensional response surface plot and contour map to deduce the optimal UF conditions. Consequently, the permeate flux was affected significantly by TMP and OT. Based on the theoretical model equation, the flux would increase as either TMP or OT increases. In reality, however, there were limitations on the achievable operating TMP and OT. Application of UF membrane at around 40°C was not recommended due to low thermal stability of the membrane, and too much pressure on the membrane and the operating apparatus is also not recommended. Furthermore, OT of the UF process generally increased by 5-6°C during the operation. Thus, optimal UF operating parameters were selected as TMP of 2.3-2.4 bar and OT of 33-34°C. The maximal permeate flux (60 L/m^2hr) was obtained at TMP of 2.1-2.5 bar and OT of 36-40°C as judged by the contour map (Fig. 2).

Macromolecules and colloidal particles easily accumulate on the UF membrane surface during the process operation, resulting in an irreversible decline of the permeate flux known as fouling. The permeate flux, in general, decreases with the operating time rapidly at the initial stage, then slower during the later stage. The effect of Sunmul temperature on the fouling index, b, which is primarily an indication of the membrane-solute interactions and fouling rates, is presented in Fig. 3. The index, b, gradually increased from 0.0303-0.0444 at 5 min process operation to 0.0705-0.0996 at 20 min. Even though the fouling effect at 40°C was lower than at 25°C during the initial 5 min operation, no significant difference in the fouling rate was detected after 10 min. On the other hand, after 20 min, the fouling at 10°C was significantly higher than those at other OTs. These results suggested that OT should be higher than 25°C to improve the separation efficiency of UF process. Therefore, the UF conditions determined based on the model equation, OT of 33-34°C and TMP of 2.3-2.4 bar, would be optimal for the efficient separation of soy-based

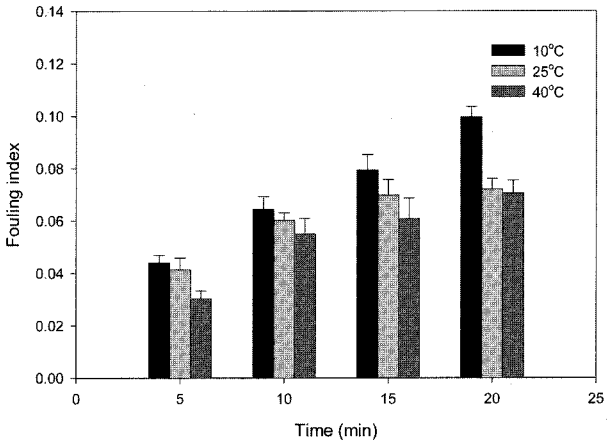

Fig. 3. Changes in fouling index at different OTs and constant of TMP (2.0 bar) from during Sunmul UF-filtration.

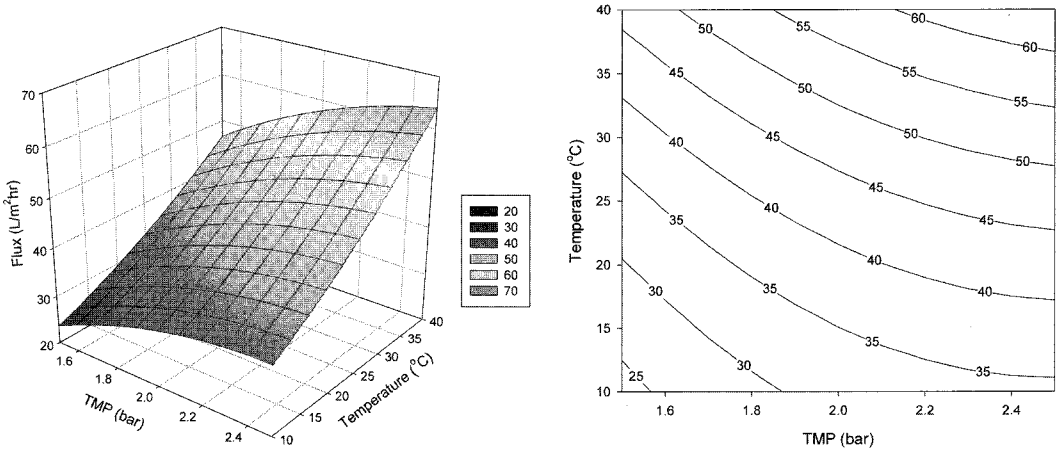

Fig. 2. Response surface and contour map on permeate flux versus OT and TMP in the process of UF-filtration.

functional materials from Sunmul.

Isoflavones and oligosaccharides in UF retentate The isoflavone and solid contents in freeze-dried UF retentate are shown in Table 2. The solid contents increased from 1.04% (w/v) of original Sunmul to 1.46-1.80% of UF retentate as VCF increased, whereas isoflavone contents steadily decreased. The amount of isoflavones concentrated by 4.0 and 10.0 VCF were 1340.89 and 1090.11 mg/g, respectively. The recovery yields of isoflavones calculated from the retentate volume and solid contents also decreased from 28.16 to 11.49% as the concentration of Sunmul increased by UF process. These results indicated that most isoflavones in Sunmul passed through the UF membrane.

The contents of oligosaccharides in Sunmul and UF retentate are presented in Table 3. Original Sunmul contained 35.4% (w/v) of oligosaccharides, and the compositional ratio of oligosaccharides was sucrose : raffinose : stachyose at 4.4 : 2.0 : 3.6. The recovery yields of oligosaccharides in Sunmul UF retentate were in the range of 12.77-27.57%, which was similar to the results of isoflavone separation. The increase in VCF allowed more of the functional compounds of isoflavone and oligosaccharides to pass through the UF membrane; therefore, further separation process by nano-filtration was adopted to recover these functional materials from the UF filtrates. Total isoflavone and oligosaccharide yields decreased by only 3% as VCF increased from 6.0 to 8.0 or from 8.0 to 10.0, whereas decreased significantly by about 10% from VCF 4.0 to 6.0. These results suggest VCF of 6.0 to be an economical process due to the fouling phenomena and filtration time.

Optimum condition of nanofiltration The response surface plot and contour map for NF process is shown in Fig 4. Similar to the procedure for UF optimization, three levels of two independent variables, OT and TMP, were selected to optimize the NF conditions. The regression

Table 2. Solid and isoflavone contents of Sunmul and UF retentate

	Sunmul	UF retentate (VCF)			
		4.0	6.0	8.0	10.0
Volume (mL)	100.00	25.00	16.67	12.50	10.00
Total solids (%)	1.04	1.46	1.60	1.76	1.80
Isoflavone (μg/g)[1)]					
Daidzein	81.42	64.56	76.06	75.42	75.90
Daidzin	551.63	422.36	368.68	337.10	323.68
Genistein	71.34	45.80	48.64	53.22	58.46
Genistin	762.90	654.31	577.26	547.56	505.69
Glycitein	33.41	21.68	23.04	23.76	23.82
Glycitin	171.10	132.18	124.58	116.52	102.56
Total	1671.80	1340.89	1218.26	1153.58	1090.11
Total isoflavone in retentate (mg)[2)]	1.74	0.49	0.32	0.25	0.20
Total isoflavone yield (%)[3)]		28.16^{a}	18.39^{b}	14.94^{bc}	11.49^{c}

[1)]g of isoflavone/g of total solids
[2)]Total isoflavone (μg/g) × total solids (%)/100 × retentate volume (mL)
[3)] $\frac{\text{Total isoflavone in UF retentate}}{\text{Tatal isoflavone in Sunmul}} \times 100$
[a,b,c]Mean value with a row followed by the different letters are significantly different at 1% level by Duncan's multiple range tests.

Table 3. Solid and oligosaccharide contents of Sunmul and UF retentate

	Sunmul	UF retentate (VCF)			
		4.0	6.0	8.0	10.0
Volume (mL)	100.00	25.00	16.67	12.50	10.00
Total solids (%)	1.04	1.46	1.60	1.76	1.80
Oligosaccharide (g/g)[1)]					
Sucrose	0.157	0.122	0.099	0.099	0.100
Raffinose	0.071	0.024	0.022	0.023	0.028
Stachyose	0.126	0.132	0.127	0.128	0.134
Total	0.354	0.278	0.248	0.250	0.262
Total oligosaccharide in retentate (g)[2)]	0.368	0.101	0.066	0.055	0.047
Total oligosaccharide yield (%)[3)]		27.57^{a}	17.93^{b}	14.95^{bc}	12.77^{c}

[1)]g of oligosaccharide / g of total solids
[2)]Total oligosaccharide (g/g) × total solids (%)/100 × retentate volume (mL)
[3)] $\frac{\text{Total oligosaccharide in UF retentate}}{\text{Tatal oligosaccharide in Sunmul}} \times 100$
[a,b,c]Mean value with a row followed by the different letters are significantly different at 1% level by Duncan's multiple range tests.

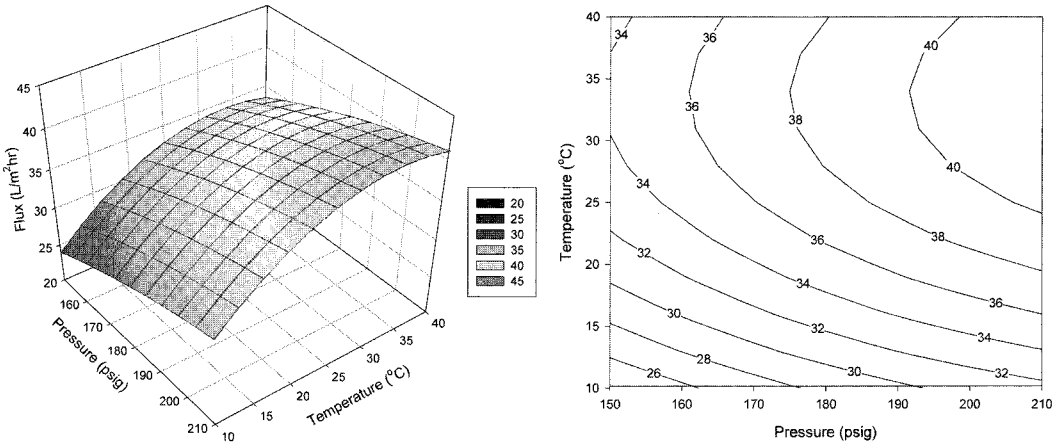

Fig. 4. Response surface and contour map on permeate flux versus OT and TMP in the process of NF-filtration.

analysis resulted in the following model equation: (Flux) = -29.511905 + 0.403571(OT) + 1.221419(TMP) - 0.000774 $(OT)^2$ - 0.018095$(TMP)^2$ (R^2=0.9938). The permeate flux was significantly affected by OT and TMP. The maximal permeate flux of 40 L/m^2hr was obtained at TMP of 192-195 psig and OT of 30-33°C.

Fouling effect of OT on the NF process was determined, displaying an overall increase in the fouling index from 0.0253-0.0425 at 5 min of NF operation to 0.0857-0.1490 at 15 min. Both UF process and the NF system showed similar increasing patterns of the fouling indices; however the fouling rate of NF process was much higher at 10°C (Fig. 5). During the NF operation, no significant difference was observed in the fouling index between 25 and 40°C at 15 min. Thus, the fouling phenomenon on the surface of nano-membrane can be reduced effectively at temperatures higher than 25°C during the NF process, and the increase in OT also improves the process efficiency. Consequently, TMP of 192-195 psig and OT of 30-33°C were selected to reduce the process operation time and the fouling on the NF membrane for optimal separation and concentration of Sunmul functional components.

Isoflavones and oligosaccharides in NF retentate NF was performed on the UF permeate to separate and concentrate the isoflavones and oligosaccharides. Table 4 shows the solid and isoflavone contents of freeze-dried NF retentate at various VCFs. The solid contents of NF retentate increased from 2.80 to 4.81% as VCF increased, whereas the total amount of isoflavones per unit weight of freeze-dried NF retentate decreased slightly. In other words, the total amount of isoflavones in NF retentate gradually decreased o 49.4% of the original Sunmul amount when VCF reached 10. This result indicated that isoflavones selectively passed through the NF permeate as the concentration process proceeded further. Starting with 100 mL of original Sunmul, the UF permeate of VCF 6.0 was 83.33 mL. When the UF permeate was further filtered through the NF membrane, the volume range of NF retentate (8.33-20.83 mL) was obtained at various VCFs of nano-filtration. The total amount of isoflavones per unit weight of dried NF retentate was not statistically different between VCF 4.0 and 6.0, while substantial decrease was observed when the UF filtrate was more concentrated. Thus, concentration up to VCF 6.0 would be reasonable

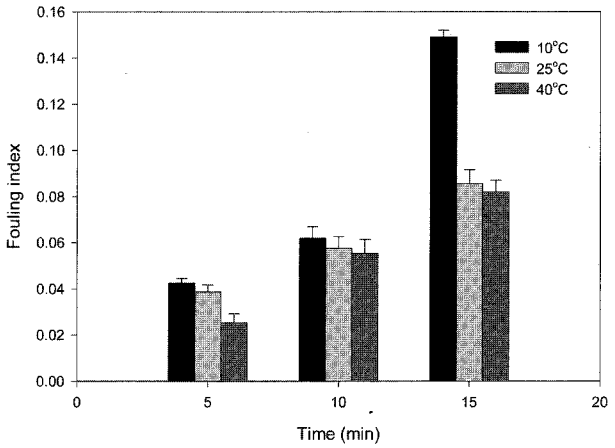

Fig. 5. Changes in fouling index at different OTs and constant TMP (150 psig) during Sunmul NF-filtration.

for achieving greater yield of isoflavones.

In the case of oligosaccharides, 46-50% (w/w) of the solids consisted of sucrose, raffinose, and stachyose, a 40 % increase from the oligosaccharide content of Sunmul (Table 5). This result indicates that most oligosaccharides remained in the NF retentate. On the basis of 100 mL of Sunmul, total oligosaccharides calculated by volume and % solids of NF retentate gradually decreased from 0. 27 to 0.20 g as VCF increased. To apply the NF separation process, the optimal level of volume decreased should be considered with losing minimum amount of the functional materials. The recovery yield of oligosaccharides from Sunmul also decreased from 72.43 to 54.44%. Because no statistical difference was observed in oligosaccharide yields between VCF 4.0 and 6.0, VCF 6.0 can be applied to the process.

These results suggest VCF 6.0 as an economic process for UF and NF to separate isoflavones and oligosaccharides in Sunmul. Consequently, to establish an effective separation process system for soy-based isoflavones and oligosaccharides, UF process can be applied to filter out macro-size molecules and the NF process should be used to recover the functional materials. In this study, 76 and 72 % of Sunmul isoflavones and oligosaccharides were recovered, respectively, from NF process. As a result of

Table 4. Solid and isoflavone contents of Sunmul and NF retentate

	Sunmul	NF retentate (VCF)			
		4.0	6.0	8.0	10.0
Volume (mL)	100.00	20.83	13.89	10.42	8.33
Total solids (%)	1.04	2.80	4.12	4.74	4.81
Isoflavone (μg/g)[1)]					
Daidzein	81.42	118.84	115.77	101.00	103.46
Daidzin	551.63	670.27	643.32	592.19	552.96
Genistein	71.34	10.24	9.80	9.10	6.68
Genistin	762.90	1260.02	1247.70	1253.97	1227.64
Glycitein	33.41	35.49	35.07	30.18	32.33
Glycitin	171.10	278.12	266.55	238.40	229.44
Total	1671.80	2372.98	2318.21	2224.84	2152.51
Total isoflavone in retentate (mg)[2)]	1.74	1.38	1.33	1.10	0.86
Total isoflavone yield (%)[3)]		79.31[a]	76.44[a]	63.22[b]	49.43[c]

[1)]g of isoflavone / g of total solids

[2)]Total isoflavone (mg/g) × total solids (%)/100 × retentate volume (mL)

[3)] $\frac{\text{Total isoflavone in NF retentate}}{\text{Tatal isoflavone in Sunmul}} \times 100$

[a,b,c]Mean value with a row followed by the different letters are significantly different at 1% level by Duncan's multiple range tests.

Table 5. Solid and oligosaccharide contents of Sunmul and NF retentate

	Sunmul	NF retentate (VCF)			
		4.0	6.0	8.0	10.0
Volume (mL)	100.00	20.83	13.89	10.42	8.33
Total solids (%)	1.04	2.80	4.12	4.74	4.81
Oligosaccharide (g/g)[1)]					
Sucrose	0.157	0.236	0.232	0.239	0.251
Raffinose	0.071	0.076	0.081	0.083	0.092
Stachyose	0.126	0.145	0.148	0.164	0.157
Total	0.354	0.457	0.461	0.486	0.500
Total oligosaccharide in retentate (g)[2)]	0.368	0.267	0.264	0.240	0.200
Total oligosaccharide Yield (%)[3)]		72.43[a]	71.69[a]	65.23[b]	54.44[c]

[1)]g of oligosaccharide / g of total solids

[2)]Total oligosaccharide (g/g) × total solids (%)/100 × retentate volume (mL)

[3)] $\frac{\text{Total oligosaccharide in NF retentate}}{\text{Tatal oligosaccharide in Sunmul}} \times 100$

[a,b,c]Mean value with a row followed by the different letters are significantly different at 1% level by Duncan's multiple range tests.

sequential UF and NF processes, 5.2 and 9.8% of initial contents were lost as NF permeates. Because the molecular weights of isoflavones and oligosaccharides were in very similar ranges, the yields of both functional materials were also very close.

COD, BOD, and SS in permeate The COD, BOD, and SS levels of original Sunmul were 8419, 4090, and 1833 ppm, respectively (Table 6). These values were 50-100 times as high as the allowance level of water pollution, which would cause a serious water pollution problem. However, as the sequential separation processes of MF, UF, and NF proceeded, the levels of COD, BOD, and SS decreased drastically. COD in Sunmul decreased from 8,419 to 114 ppm after three sequential filtrations, indicating that COD was decreased by more than 98% through this operation. The MF and UF processes reduced BOD by over 90%, from 4,090 to 398 ppm. SS was not detected after the UF process. These values were comparable to the standard allowance level of water pollution, that is, 70-90 ppm of COD and 60-80 ppm of BOD. Therefore, the filtration processes were not only useful for separation of isoflavones and oligosaccharides from Sunmul, but were also effective for reducing water contaminants.

Table 6. COD, BOD, and SS in the permeates after MF-, UF-, and NF-filtration of Sunmul (unit: ppm)

	Sunmul	Permeate		
		MF	UF	NF
COD	8,419	7,428	5,292	114
BOD	4,090	2,600	398	77
S S	1,833	174	ND	ND

ND : not detected

Acknowledgments

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