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Purification of Soybean Oligosaccharides of Tofu Whey Using a Hybrid Process Based on Enzymatic Hydrolysis and Nanofiltration

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ABSTRACT

Tofu whey, liquid waste of tofu processing, contains valuable components including protein, mono- and di-saccharide (glucose, fructose, sucrose, MDS) and soy oligosaccharide (stachyose, raffinose, SOS). Recovery of these components, therefore, is essential regarding sustainable food processing approach. Ultrafiltration (UF) has been successfully used for concentration of tofu whey protein. In this study, nanofiltration was employed to harvest and purify SOS from tofu whey UF-permeate. NF-membrane selection, hydrolysis of tofu whey UF-permeate and NF of pre-hydrolysis of tofu whey UF- permeate were performed to achieve the best condition for purification. It was found that the membrane, NF-DK-2540 was superior than that of NF-DL-2540 with the retention rate of stachyose (94%), raffinose (87%), sucrose (65%), glucose (7.8%) and fructose (4.3%). These results indicated that NF could harvest SOS and remove monosaccharide with acceptable retention. However, its sucrose retention was considerably high. Therefore, an enzymatic hydrolysis of UF-permeate was employed to convert sucrose to glucose and fructose. The results indicated that the use of NF combined with enzymatic hydrolysis could significantly remove MDS from tofu whey UF-permeate and consequently increasing the purity of SOS.

Keywords: Nanofiltration, enzymatic hydrolysis, oligosaccharide, oligosaccharide purification, tofu whey

1.0 INTRODUCTION

Tofu whey is considered as liquid waste of tofu processing plant usually discharged to wastewater treatment system. It contains valuable components including proteins, glucose, fructose and sucrose (mono- and disaccharide, MDS) as well as stachyose and raffinose (soy oligosaccharides, SOS). This SOS is considered as functional food ingredient due to its prebiotic property and it is approved as safe ingredient [1, 2]. Nowadays, most of commercial SOS available in the market is directly extracted from soybean seed, using either water or chemicals as solvent [3]. Regarding bio-circular-green economy model (BCG) therefore, producing SOS from tofu whey is not only an excellent way of full utilization of waste but also reduction of waste for wastewater treatment system.

To harvest these valuable components, ultrafiltration (UF) with pH-shifting method has been successfully employed to recover and concentrate protein (UF-retentate) from tofu whey while SOS presented in the tofu whey UF-permeate is of interest [4]. Therefore, process development for harvesting and purifying SOS from the tofu whey UF-

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permeate is crucially required.

Nanofitration (NF) is a pressure-driven membrane process. Its molecular weight cut off (MWCO) and operating pressure are laid in between UF and reverse osmosis (RO). Mass transported across the NF membrane is dominated by convection and/or diffusion [5]. Regarding its rejection characteristic, NF has a capability to fractionate monovalent, multivalent ions and organic solutes such as enzymes as well as saccharides from a multi-component mixture [6, 7]. NF has been successfully employed in various industries. The oligosaccharide can be separated from a multi component mixture containing mono- and di-saccharides using different types of NF membrane [6, 7, 8]. These studies suggested that the retentions of disaccharide (e.g. lactose) and monosaccharide (e.g. glucose, fructose), oligosaccharide (e.g. fructo- and galacto-oligosaccharide) were influenced by the type of membrane, operating temperature and transmembrane pressure (TMP). In term of separation ability, most of oligosaccharide was retained by NF membrane while most of monosaccharide was removed from the feed. A hybrid process based on UF and NF has been applied in the food industry. For instance, in production of oligosaccharide from chicory extract, UF is used to remove large particles and macromolecules e.g. proteins, starches, insoluble substances while the NF is used to concentrate oligosaccharide [9]. In cheese processing, UF and NF are usually used to increase cheese yield. The major components of cheese whey are proteins, lactose and ash. Whey protein can be recovered and concentrated by UF while lactose, presented in UF-permeate can be concentrated by NF [10, 11].

Generally, the retention of NF membrane is influenced by several factors, including operating conditions, feed and membrane characteristics. Pretreatment of the feed may be required particularly in the case of purification of oligosaccharide from the feed containing both mono- and di-saccharides. A few studies had indicated that disaccharide retention of NF membrane is higher than 80%. For example, sucrose retention during crossflow NF of sugar mixture solution is about 99% [6]. In purification of sugar beet press juice, the retention of sucrose of NF membrane is about 99.7% [12]. These results indicate that the use of NF alone is not able to purify oligosaccharides from mixture solution containing disaccharides. Recently, high purity of galacto-oligosaccharide has been developed by depleting glucose and lactose from galacto-oligosaccharide syrup by selected nonconventional yeast fermentation [13]. However, separation process for yeast removal is required. For purification of oligosaccharide from tofu whey UF-permeate, an enzymatic hydrolysis therefore possibly required to convert disaccharides to monosaccharides to be able to remove by NF.

This study aimed to develop the suitable process for purification of SOS produced from tofu whey UF-permeate. NF membrane was carefully selected regarding its ability in SOS and MDS separation. Enzymatic hydrolysis and NF were combined as a hybrid process to increase the purity of SOS. The effect of enzyme unit to substrate ratio (E:S) on sucrose conversion was studied. The effect of operating conditions during NF of hydrolyzed tofu whey UF-permeate on permeate flux and SOS and MDS retentions were also investigated.

2.0 MATERIAL AND METHODS

2.1 Materials

2.1.1 Preparation of Tofu Whey and UF-permeate

Tofu whey was prepared by traditional method according to Kao *et al.* [14]**.** The tofu whey UF-permeate was prepared using a pilot scale system. The UF tubular module (Rhodia, KERASEP^{TM}, Orelis, France) with MWCO 50 kDa was used. The system was operated at TMP, crossflow rate (CFR) and temperature of 5 bar, 2000 L.h^{-1} and 50 ºC, respectively.

2.1.2 Invertase Enzyme

A commercial food grad invertase (Maxinvert® 200000MG), produced by a selected strain of *Saccharomyces cerevisiae*, was purchased from DSM Food Sp. (Seclin, France). The activity declared by the manufacturer was 200,000 SU.g^{-1}.

2.2 Methods

The experiment study including selection of NF-membrane, hydrolysis of sucrose and nanofiltration was setup and shown in Figure 1.

2.2.1 NF Membrane Selection

Two types of NF spiral-wound, thin film membrane (DK-2540 and DL-2540) were used (GE Water & Process Technology, USA). According to membrane manufacturing information, the retention of $MgSO_4$ of DK-2540 (98%) is slightly higher than that of DL-2540 (96%). The experiments were carried out using a pilot scale system and operated under total recycle mode at CFR of 500 Lh^{-1}, TMP of 4 bar and temperature of 20 ºC. The content of glucose, fructose, sucrose, stachyose and raffinose in the feed and permeate samples were analyzed using high performance liquid chromatography (HPLC). The suitable membrane was selected according to SOS and MSD retentions.

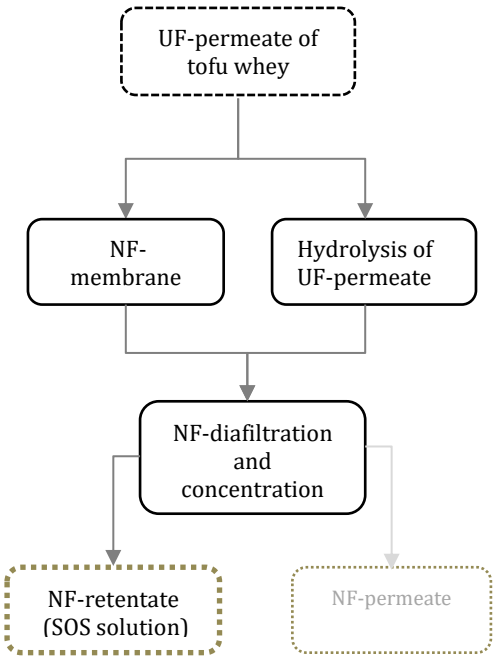

Figure 1 Experimental setup for SOS purification study using tofu whey as feed material

2.2.2 Enzymatic Hydrolysis

In this study, the tofu whey UF-permeate whey was hydrolyzed with invertase aiming to convert sucrose to monosaccharides. The E:S ratios (1, 5 and 10 Uml^{-1} of sample) were used for hydrolysis at pH 6.5 and temperature of 45 ºC. The suitable E:S ratio for hydrolysis was selected according to its ability in converting sucrose to glucose and fructose.

2.2.3 Effect of Cross Flow Rate (CFR) and Transmembrane Pressure (TMP) of NF on Permeate Flux and Saccharide Retention

The tofu whey UF-permeate, hydrolyzed at 45 ºC using E:S ratio of

5 Uml^{-1} for 30 min, was used for NF. The effect of CFR (400-600 $L.h^{-1}$) and TMP (6-10 bar) on permeate flux and saccharide retention, operated under total recycle mode using NF (DK-2540) membrane were studied. The permeate flux and saccharide retention were used to select the optimum operating condition.

2.2.4 Removal of Low Molecular Weight Sugar

The experiments were performed with discontinuous diafiltration. The pre-hydrolysed tofu whey UF-permeate sample was diluted by adding water about 2 times of the initial feed volume. The sample was fed to the membrane system, operated at TMP of 6 bar, CFR of 600 $L.h^{-1}$ and temperature of 20 ºC. The permeate was continuously collected and the retentate was recycled to the feed tank. The experiment was performed to achieve concentration factor (VCF) of 9. The permeate and retentate samples were taken for analysis of SOS and MDS content.

The permeate flux ($Lm^{-2}h^{-1}$) was calculated using the following equation;

$$J = V/At \quad (1)$$

where V is the permeate volume(L), A is the membrane effective area(m^2) and t is the operating time(h).

The saccharide retention(R) was calculated using the following equation;

$$R = 1\text{-} (C_p/C) \quad (2)$$

where C_p and C are the concentration of saccharide in the permeate and feed, respectively.

The volume concentration factor (VCF) was calculated using the following equation;

$$VCF = V_0/ (V_0\text{-}V_p) \quad (3)$$

where V_0 is the initial feed volume and V_p is the collected permeate volume.

2.2.4 Analytical Methods

The saccharide content in the feed and permeate was analyzed using HPLC (Rezex™, RNM-Carbohydrate Na+ 8%, 300 mm x 7.8 mm, Phenomenex, USA) at a flow rate of 0.4 $mlmin^{-1}$ and temperature of 80ºC.

3.0 RESULTS AND DISCUSSION

3.1 NF Membrane Selection

Two types of NF membrane were tested using tofu whey UF-permeate as a feed**.** Retentions of saccharides were used to determine their ability of separation. Figure 2. presents the retention of SOS (stachyose, raffinose) and MDS (sucrose, glucose and fructose) of DK-2540 and DL-2540 membranes**.** It was found that the retentions of stachyose, raffinose and sucrose for DK-2540 membrane were 94.1, 87.4 and 81.0%, respectively. while the retention of glucose and fructose were relatively low at 7.8% and 4.4%, respectively. This result was similar to those using cellulose acetate NF membrane [6]. For DL-2540 membrane, the retentions of stachyose and raffinose were just slightly higher than 60% while the retentions of sucrose and fructose were less than 40% which is similar to those thin film composite membrane (DS-GE, class UF) [6]. It is worthy to note that the retention of glucose for DL-2540 membrane was 0 %. The difference in retention obtained from these membranes could be due to the difference in membrane characteristics as indicated using $MgSO_4$ rejection**.** These results indicate that the DK-

2540 membrane was more suitable for harvesting SOS and removing monosaccharide compared with DL-2540 membrane. Since the NF DK-2540 membrane could not effectively remove sucrose, additional process to transform sucrose into glucose and fructose is required.

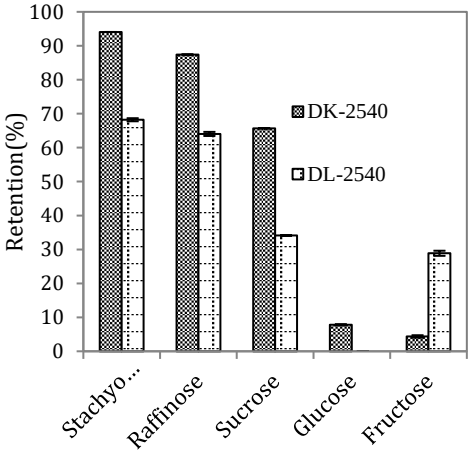

Figure 2 Retention values for stachyose, raffinose, sucrose, glucose and fructose during nanofiltration of tofu whey UF-permeate

3.2 Enzymatic Hydrolysis

In order to reduce sucrose in the finish product, sucrose in the tofu whey UF-permeate was hydrolysed by the inverstase enzyme to obtain fructose and glucose which are able to be removed by DK-2540 membrane. Figure 3. shows sucrose and soy oligosaccharide concentration during hydrolysis of tofu whey UF-permeate as varying ratio of enzyme unit to sample volume (E:S). Sucrose concentration decreased sharply at hydrolysis time of 30 min. Increasing hydrolysis time for 60 and 90 min did not significantly reduce sucrose concentration. It was also observed that the hydrolysis time and ratio of enzyme to sample volume slightly affected soy oligosaccharides concentration.

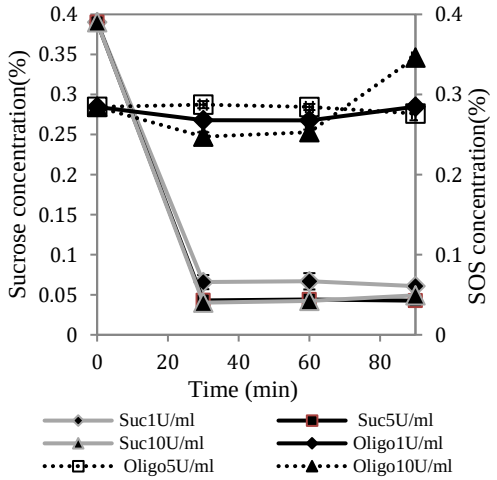

Figure 3 Soy oligosaccharide (SOS) concentration during hydrolysis of tofu whey UF-permeate at pH 6.5, 45 ºC as varying enzyme unit to substrate ratio (E:S ratio)

The oligosaccharide concentration, hydrolysed using E:S ratio of 10 Uml^{-1}, slightly decreased from the initial value. The invertase can hydrolyze sucrose (D-glucopyranose and D-fructofuranose) by splitting of α-1, 4-glycosidic bonds, obtaining invert sugars, glucose and fructose [15]. In addition, stachyose is non-reducing tetrasaccharide (2 D-galactopyranose, D-glucopyranose and D-fructofuranose) while raffinose is a trisaccharide (D-galactopyranose, D-glucopyranose and D-fructofuranose), respectively. These oligosaccharides can also be converted to monosaccharide by invertase [16, 17]. Soy oligosaccharide concentration of the sample, hydrolysed using E:S ratio of 10Uml^{-1} for 90 min, increased approximately 1.2 times. It was probably due to the result of transglycosidation reaction by invertase. Regarding sucrose concentration reduction, hydrolysis of tofu whey UF-permeate at pH 6.5, temperature of 45 ºC using E:S ratio of 5 Uml^{-1} for 30 min was determined as optimum condition.

3.3 Effect of CFR and TMP of NF on Permeate Flux and Saccharide Retention

A pilot plant nanofiltration system using DK-2540 membrane was employed to harvest soy oligosaccharide and to remove glucose and fructose from tofu whey UF-permeate, hydrolysed with invertase using E:S ratio of 5 Uml^{-1} at 45 °C for 30 min. The effect of CFR and TMP on permeate flux under total recycle mode is shown in Figure 4. The result shows that the permeate flux increased with CFR and TMP. It is worthy to note that the feed of tofu whey UF-permeate also contained small amount of soluble protein.

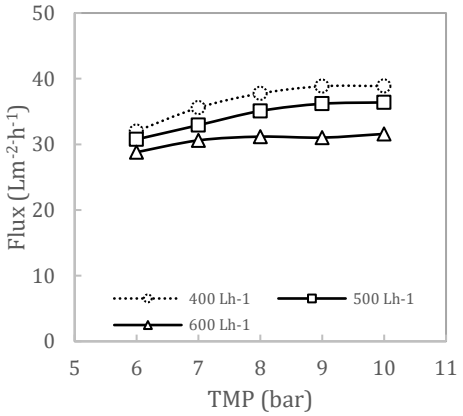

Figure 4 Effect of crossflow rate and transmembrane pressure on permeate flux during nanofiltration of hydrolysed tofu whey UF-permeate

When the permeate flux increased with TMP, concentration of solutes on the membrane surface would accrue to the certain value in which protein can form gel layer [10,18]. The permeate flux during NF of this feed sample increased as TMP increased. All permeate fluxes were almost stable at TMP 9 bar. These results indicated that the permeate flux was in mass transfer control region. The limiting fluxes (at TMP 9 bar) operated at CRF of 400, 500 and 600 Lh^{-1} were approximately 32, 36 and 38 $Lm^{-2}h^{-1}$, respectively. It is known that an increase in CFR led to increase in mass transfer, consequently increase permeate flux [19].

Table 1 presents fructose, stachyose and raffinose retentions as varying TMP and CFR. In general, the retention of fructose and soy oligosaccharide increased with TMP and CFR. Similar results were found during NF of raffinose, sucrose and fructose model solution [6]. At CFR of 600 Lh^{-1} and TMP 6 to 10 bar, stachyose and raffinose were completely retained while higher than 50% of fructose was rejected. At CFR of 500 and 400 Lh^{-1}, stachyose, raffinose and fructose retentions were less than those at 600 Lh^{-1}. It has been reported that the concentration of each sugar in saccharide mixture solution also affected the solute flux [6]. In this study, the retention of stachyose was less than that of raffinose because concentration of stachyose after hydrolysis was higher than raffinose. As a result, the solute flux of stachyose was higher than that of raffinose. It important to note that sucrose and glucose retention concentration in the permeate were less than 0.01 % (wv^{-1}) which is too low to be detected by HPLC. When operating TMP elevated, concentration of the solute on membrane surface would also be accrued. It caused slow accreting of solute permeate flux due to diffusion. Meanwhile, the solvent permeate flux increased in direct proportion with operating pressure that much larger than solute permeate flux, resulting in higher retaining rate [6, 19, 20]. In addition to TMP, CFR also had great effect on saccharide retention. Stachyose and fructose retentions were significantly increased with CFR. When CFR was greater, concentration polarization and accumulation of rejected solute on the membrane wall would be decreased due to an increase in back transportation.

Regarding these results, operating at CFR of 600Lh^{-1} and TMP of 6 bar was chosen for further study.

Table 1 Soy oligosaccharides and fructose retentions (%) as varying transmembrane pressure (TMP) and crossflow rate (CFR) during nanofiltration of pre-hydrolysed tofu whey UF-permeate

Stachyose					
	TMP(bar)				
CFR(Lh^{-1})	6	7	8	9	10
600	100.00±0.00^{ns,A}	100.00±0.00^{ns,A}	100.00±0.00^{ns,A}	100.00±0.00^{ns,A}	100.00±0.00^{ns,A}
500	81.80±0.10^{d,B}	82.52±0.17^{c,B}	83.63±0.37^{b,B}	84.47±0.12^{a,B}	84.79±0.17^{a,B}
400	65.05±0.41^{c,C}	67.25±0.82^{b,C}	67.54±0.66^{ab,C}	68.29±0.25^{ab,C}	68.52±0.61^{a,C}
Raffinose					
	TMP(bar)				
CFR(Lh^{-1})	6	7	8	9	10
600	100.00±0.00^{ns,NS}	100.00±0.00^{ns,NS}	100.00±0.00^{ns,NS}	100.00±0.00^{ns,NS}	100.00±0.00^{ns,NS}
500	100.00±0.00^{ns,NS}	100.00±0.00^{ns,NS}	100.00±0.00^{ns,NS}	100.00±0.00^{ns,NS}	100.00±0.00^{ns,NS}
400	100.00±0.00^{ns,NS}	100.00±0.00^{ns,NS}	100.00±0.00^{ns,NS}	100.00±0.00^{ns,NS}	100.00±0.00^{ns,NS}
Fructose					
	TMP(bar)				
CFR(Lh^{-1})	6	7	8	9	10
600	56.78±0.95^{c,A}	57.08±1.72^{c,A}	58.84±0.64b^{c,A}	60.78±0.48^{b,A}	65.44±0.71^{a,A}
500	45.65±0.69^{e,B}	46.96±0.00^{d,B}	51.38±0.35^{c,B}	55.57±0.64^{b,B}	63.15±1.52^{a,A}
400	22.47±0.09^{d,C}	36.48±0.92^{c,C}	46.89±2.18^{b,C}	47.87±1.71^{b,C}	55.92±0.23^{a,B}

Note: Each value is mean of triplicate ± SD. Different small letters in the same row indicate the significant difference ($p<0.05$) and Different capital letters in the same column indicate the significant difference ($p<0.05$)., ns,NS = non-significant difference ($p>0.05$)

3.4 Removal of Low Molecular Weight Sugar

The hydrolysed tofu whey UF-permeate was diluted with water about 2 times of its initial volume**.** Then, the diluted-hydrolysed UF-permeate was fed into the NF system, operated under batch concentration mode at CFR of 600 Lh^{-1}, TMP of 6 bar and temperature of 20 °C. The results of permeate flux and saccharides retention are shown against VCF in Figure 5. The initial permeate flux was 32 Lm^{-2}h^{-1}. The permeate flux decreased sharply until VCF of 3 was achieved and slightly decreased when VCF greater than 3. The permeate flux descended because of fouling, concentration polarization as well as an increase in osmotic pressure of saccharides. The average retentions of saccharides were in order of 100, 81 and 60 % for raffinose, stachyose and fructose, respectively. The retentions of stachyose and fructose slightly increased with VCF in the earliest stage of filtration and almost constant later. It has been pointed out that an increase in feed concentration caused a decrease in sugar retention [6, 19, 20]**.** Generally, increase in feed concentration leads to decrease back diffusion of solute and consequently increasing of solute concentration at the membrane surface. As concentration gradient across the membrane increase, the solute flux is expected to increase. In this study however, an increase in retention of

stachyose and fructose at VCF lower than 3 was probably due to fouling layer of accumulated protein [11, 18, 21].

The contributed percentage of stachyose, raffinose, sucrose, glucose and fructose calculated based on total sugar content found in samples are shown in Table 2. For tofu whey UF - permeate, sucrose contributed the highest followed by stachyose, raffinose, fructose and glucose, respectively. After hydrolysis of tofu whey UF-permeate, the stachyose contributed the highest followed by fructose, glucose, raffinose and sucrose. This could be due to the conversion of sucrose into glucose and fructose during enzymatic hydrolysis. After nanofiltration, stachyose contribute the highest followed by raffinose, fructose, glucose and sucrose.

These results indicated a hybrid process based on enzymatic hydrolysis of tofu whey UF-permeate and nanofiltration was an effective process for removal of sucrose, fructose and glucose from soy oligosaccharide mixture.

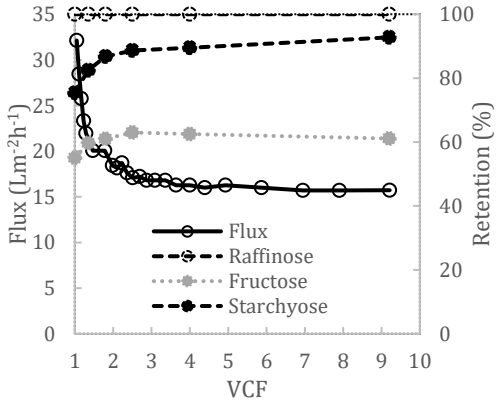

Figure 5 Permeate flux and retentions of raffinose, stachyose and fructose against volume concentration factor during nanofiltration of pre-hydrolysed tofu whey UF-permeate

Table 2 Contributed percentage of stachyose, raffinose, sucrose, fructose and glucose to total sugar before and after enzymatic hydrolysis of tofu whey UF-permeate and nanofiltration

Sample	Stachyose (%)	Raffinose (%)	Sucrose (%)	Glucose (%)	Fructose (%)
Tofu whey UF-permeate	36.7	8.6	44.6	2.3	7.7
After hydrolysis	29.9	12.8	7.0	21.2	29.1
After nanofiltration	53.9	25.1	1.0	1.0	18.9

4.0 CONCLUSION

Tofu whey UF-permeate discharged during harvesting protein from tofu whey using ultrafiltration was used as feed material for producing soy oligosaccharide with low content of mono- and di- saccharide. Series of experimental studies were designed and carried out. Two types of NF membrane were tested and selected based on ability in raffinose, stachyose retention, and sucrose, fructose, glucose permeation. The DK-2540 membrane was the most suitable for soy oligosaccharide recovery and mon- and di-saccharide removal. Enzymatic hydrolysis of tofu whey UF-permeate study results indicated that the optimum condition based on ability in converting sucrose into fructose and glucose was E:S ratio of Uml^{-1}, pH 6.5, temperature of 45 °C and hydrolysis time of 30 min**.** The operating

condition for NF of hydrolysis of tofu whey UF-permeate using spiral wound module equipped with DK 2540 membrane was CFR of 600 Lh^{-1} and TMP of 6 bar. The hybrid process based on enzymatic hydrolysis and NF was a promising process for soy oligosaccharide recovery and sucrose, fructose, glucose removal, consequently increasing the purity of soy oligosaccharide.

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