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FRACTIONATION OF PROTEINS IN SURIMI WASTE WATER USING MEMBRANE FILTRATION

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Abstract. The study was conducted to determine the feasibility of using ultrafiltration and microfiltration for fractionation of proteins in surimi waste water. The results from this study showed that the molecular weight of the soluble proteins in surimi waste water was in the range of 10-120 kDa. Ultrafiltration surimi waste water with using membrane with MWCO 100 and 300 kDa could not fractionate these proteins since most of the proteins were retained in retentate. This result suggested that fouling formed during ultrafiltration played an important role in determination of the membrane selectivity. Improving the fouling problem may be the key factor enhancing membrane selectivity. Fractionation of proteins from this waste by using microfiltration with membranes pore size 0.22, 0.45, and 1 μm was also studied. Although some proteins could penetrate through the membrane, the results from SDS-PAGE showed that the protein profiles in the retentate and permeates did not differ, indicating that these membranes also could not be used for fractionation. This may be due to the large pore size of the membranes and the narrow range of the molecular weight of these proteins.

Keywords: Surimi waste water, protein recovery, fractionation, ultrafiltration, microfiltration

1.0 INTRODUCTION

Surimi is a Japanese term of washed and dewatered fish mince widely used as a raw ingredient in manufacturing of artificial crab meats. Generally, surimi production can be divided into 7 steps, as shown in Figure 1. The processes involve extensive washing of minced fish to remove fat and water soluble substances such as sacroplasmic proteins, pigment, enzymes, and vitamin. As a result of the washing, large volumes of waste water containing high concentrations of organic materials are generated in the downstream of washing and dewatering operation. The direct discharge of the waste water from surimi industry generates negative impacts on the environment [1]. The volume of the surimi waste water discharged from the processing plant is approximately 30 liters of water per 1 kg of surimi product [2]. The wash water is generally discarded

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back into the plant's waste stream. As a result, most of the valuable components especially soluble proteins are lost [3]. However, some enzymes and soluble proteins could be highly functional proteins. Therefore, it will be beneficial to recover and fractionate those proteins.

Membrane filtration is one of the methods that has a great potential for concentration, fractionation, and purification of soluble and insoluble materials in seafood product [4]. When small quantities of proteins need to be fractionated, techniques such as chromatography, affinity separation, and electrophoresis can be used quite effectively. However, in large number of cases, greater quantities of protein solution need to be fractionated. The membrane filtration process is a fractionation technique, potentially to be used for large-scale applications [5].

Membrane applications in the seafood industry are just beginning to emerge, but an increase in the number of published studies and registered patents hints a significant development in the coming year and they can be suitable for recovery of its protein content. Due to recent technological developments, microfiltration and ultrafiltration have been used successfully for recovery of soluble and non-soluble proteins from surimi washing water [1,2]. The proteins recovered by microfiltration is mainly

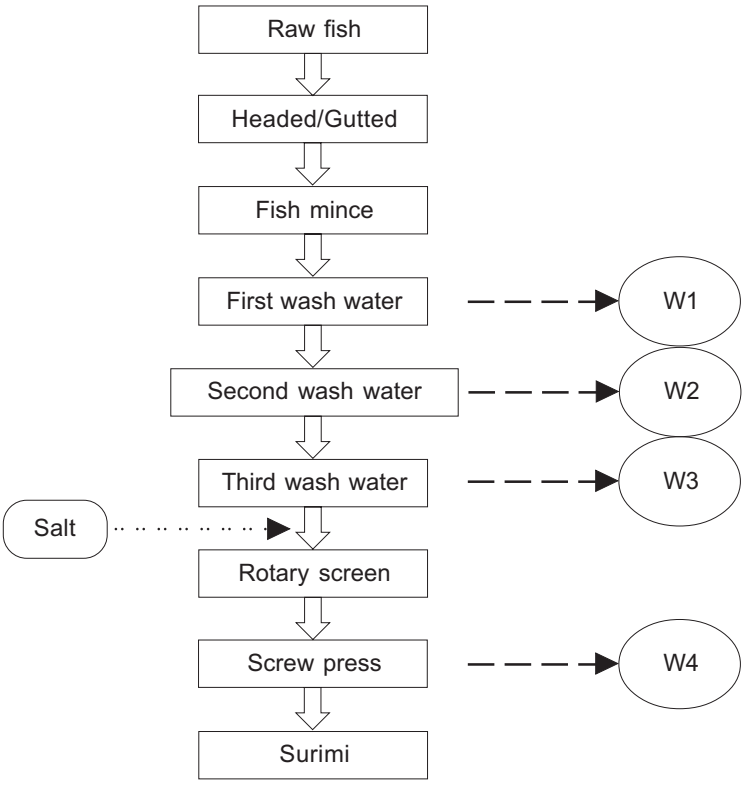

Figure1 Flow process diagram of surimi production

myofibrillar. It shows highly functional properties and composition comparable to the proteins in regular surimi. Therefore, it could be directly added to surimi to increase the yield without affecting its quality. The soluble substances recovered by ultrafiltration are a mixture of water soluble proteins and enzyme [6]. However, two major problems inherent with microfiltration and ultrafiltration are: (1) irreversible membrane fouling, which results in low permeate flux and changes membrane selectivity, and (2) membrane flow channel blockage, due to increased retentate viscosity [7]. The aim of this work is to study the feasibility of using ultrafiltration and microfiltration to fractionate soluble proteins discharged from surimi wastewater.

2.0 MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Four types of surimi washing water discharged from commercial surimi processing plant using big-eye snapper fish as raw material were used for this study. The sample of washing water were: the first washing water (W1), the second washing water (W2), the third washing water (W3), and wastewater discharged from de-watering process using screw press (W4). Note that the flow process chart of surimi processing is shown in Figure 1. The temperature of all samples was kept at 4°C and used within 24 hour after collecting. All samples were pre-filtered to remove suspended solid before being used [8].

2.1.1 Determination of Soluble Protein Content and Total Solid

The total soluble protein was determined by biuret method and the total solid was determined according to AOAC (1990).

2.2 Determine the Molecular Weight of Protein

SDS-PAGE (non β-mercaptoethanol) was used to determine the molecular weight of surimi wastewater proteins. Samples were mixed with the sample buffer with a ratio of 1:3 and loaded to SDS-substrate gel (gradient 4-20%). The gel was stained in 0.125% coomassie blue R-250, destained in a 50% methanol, and 10% acetic acid solution followed by a 5% methanol and 7% acetic acid solution (modified by [9]). Molecular weight standards (Promega V8491) were used for the estimation of apparent molecular weight of the protein bands.

2.3 Membrane Filtration Process

Only W1 and W4 samples were used for the filtration study since their impurity is higher than the others. For ultrafiltration, the plat and frame of regenerated cellulose

membranes with molecular weight cut off (MWCO) 100 and 300 kDa were used (Millipore). The experiment was ran in a batch mode at a constant transmembrane pressure (TMP) and temperature, 2.5 bar and 8±k2°C respectively. It was ended when the concentration factor was 10 folds. During the experiment, the permeate flux (J) was measured and the sample of permeate was collected at the end of each run for chemical analysis. For microfiltration, the membranes used were cellulose acetate with pore size of 0.22, 0.45, and 1 μm. The experiment was performed at constant TMP and temperature of 2.5 bar and 7±2°C respectively. The permeation flux (J) was investigated and ended when the steady flux was obtained. The permeate sample then was collected for the chemical analysis.

3.0 RESULTS AND DISCUSSION

3.1 Composition Profile of Surimi Waste Water

The physical and chemical properties of waste water discharged from washing and dewatering stages were shown in Table 1. Their pH were in the range of 6.8 to 7.1. The protein content and total solid in the waste water were in the rage of 1.1 to 4.20 mg/ml and 589 to 854 mg/l respectively. The waste discharged from the dewatering (W4) contained the highest level of protein and the total solid while washing water (W3) contained the lowest level of protein. The protein content in W2, W3, and W4 were similar to those reported in the literature [2]. However, the protein content for W1 was much lower than those reported. This could be due to the sample used in this study which was prefiltered before used and large suspension particles such as pieces of mince fish, containing in the sample which were removed. Result from SDS-PAGE (Figure 2) study showed that the molecular weigh of protein for all samples varied from 10 to 100 kDa. This result was similar to those in the literature [2].

Table 1 Physical and chemical properties of surimi waste water

Sample	pH	Protein (mg/l)	Total solid(mg/l)
W1	6.8	1.57±0.19	4.20±0.35
W2	7.1	1.03±0.16	3.20±0.43
W3	6.9	0.11±0.03	1.14±0.11
W4	6.8	5.53±0.26	6.42±0.24

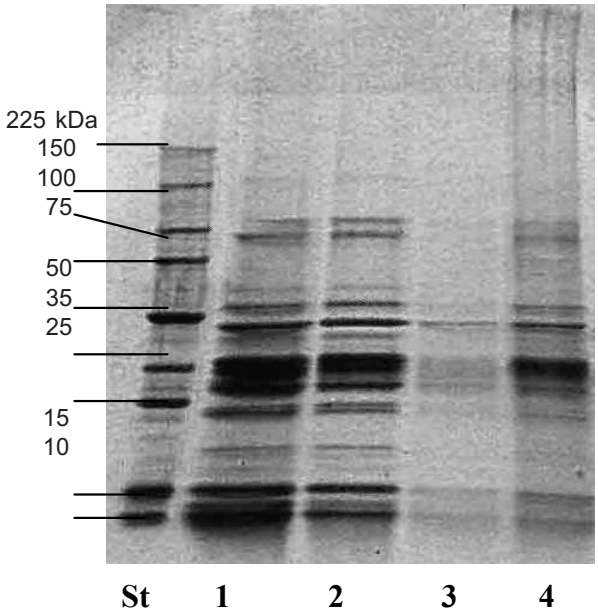

Figure 2 SDS-PAGE pattern of surimi wastewater fractionlane1:W1 lane2:W2 lane3:W3, lane4:W4 and St: standard protein.

3.2 Permeate Flux Profile

The permeate fluxes during microfiltration and ultrafiltration of W1 and W4 were shown in Figures 3 and 4. For both microfiltration and ultrafiltration, there were a substantial decrease in the initial flux during the first few minutes after apparent steady state equilibrium. In general, the steady flux of both microfiltration and ultrafiltration of W1 were higher than those of W4. This could be due to the difference in protein concentration. Flux decline during microfiltration and ultrafiltration of protein solution could be due to fouling and concentration polarization. It was found that this decrease in initial permeate flux during microfiltration and ultrafiltration was due to the internal fouling, especially pore blocking, and the subsequent decline was related to the boundary layer near the membrane surface and the cake layer deposited on the membrane surface [7]. It has been reported that fouling during microfiltration and ultrafiltration also changed the membrane selectivity.

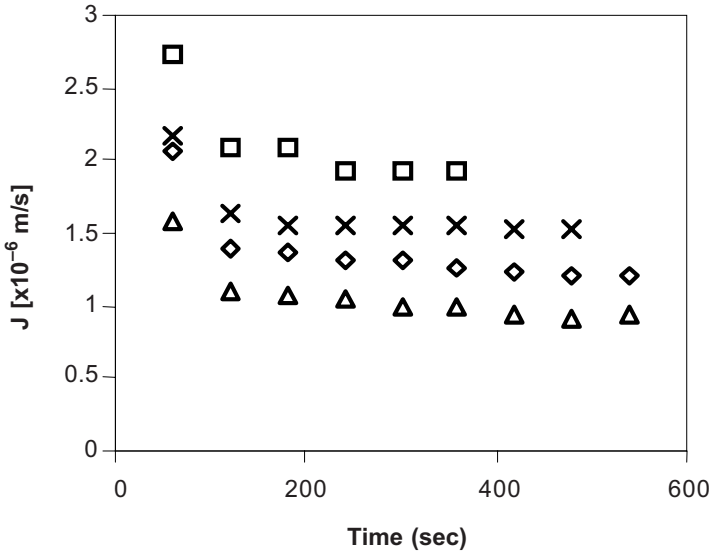

Figure 3 Permeate flux during ultrafiltration of surimi waste waster at 8±2°C, TMP 2.5 bars: W1/100 kDa (◇), W1/300 kDa (□),W4/100 kDa (△), W4/300 kDa (×)

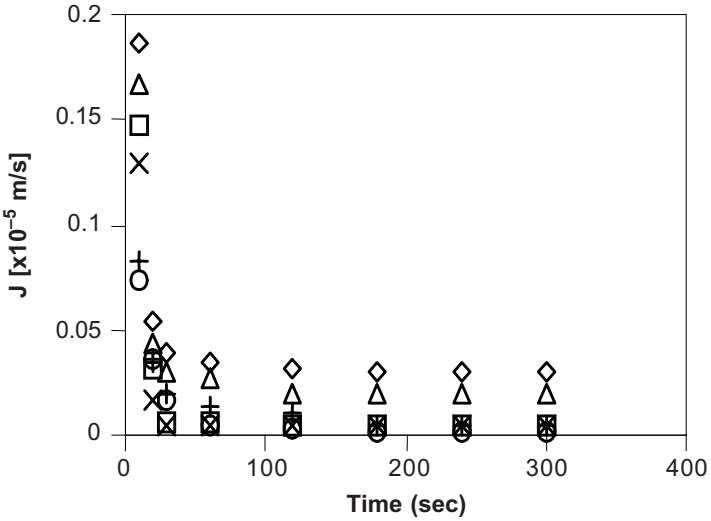

Figure 4 Permeate flux during microfiltration of surimi waste water at 8+2 °C, TMP 2 bars: W1/1 µm (◇), W1/0.45 µm (△), W1/0.22 µm (+), W4/1 µm (□), W4/0.45 µm (×), W4/0.22 µm (○).

3.3 Protein Transmission

Protein transmission or membrane selectivity is one the key factors that can be used for determination of the feasibility using microfiltration and ultrafiltration for protein fractionation. The result of protein transmission of all samples was shown in Table 2.

Table 2 Protein transmission during ultrafiltration and microfiltration of surimi waste water

Sample / (MWCO or pore size)	Protein concentration (mg/l)	
	Retentate	Permeate
W1/(UF 100 kDa)	13.47 ± 1.60	ND
W1(UF 300 kDa)	12.63 ± 1.15	ND
W4(UF 100 kDa)	26.66 ± 0.81	ND
W4(UF 300kDa)	26.38 ± 0.95	ND
W1(MF 0.22 µm)	7.46 ± 1.55	0.26 ± 0.05
W1(MF 0.45 µm)	5.74 ± 0.77	1.34 ± 0.05
W1(MF 1 µm)	4.81 ± 0.18	1.86 ± 0.05
W4(MF 0.22 µm)	8.09 ± 0.60	0.16 ± 0.05
W4(MF 0.45 µm)	7.64 ± 0.40	1.27 ± 0.05
W4(MF 1 µm)	7.26 ± 0.31	5.51 ± 0.05

W1 and W4: surimi waste water as illustrated in Figure 1.
ND: not detected

In general, all proteins from W1 and W4 were totally rejected by ultrafiltration using 100 and 300 kDa membranes, while some proteins could penetrate through microfiltration membranes. These results suggested that ultrafiltration could not be used to fractionate protein in W1 and W2. The result of ultrafiltration study was unexpected since the molecular weight of protein in W1 and W2 was in the range of 10 to 100 kDa, which is fairly smaller than the MWCO of the membranes, and some proteins, especially low molecular weight protein should penetrate through the membrane. These could be due to severe fouling during ultrafiltration. This fouling should be located in the membrane pore (internal fouling and/or gel layer formed on the membrane surface (external fouling). As a result, the selectivity of membrane was changed. The selectivity during ultrafiltration of mixture protein solution has been studied intensively [5]. Their results suggested that the membrane selectivity was depending on membrane property and the operating condition, especially the crossflow velocity and permeates flux. These parameters are closely linked to membrane fouling and transportation mechanism of the proteins. To improve membrane selectivity, the effect of these parameters on membrane selectivity must be studied.

For microfiltration, there was a significant amount of protein which could penetrate through the membrane (Table 2). It was found that increasing membrane pore size will increase protein transmission. A similar protein profile in the retentate and permeate was found for all samples (Figure 5). This result indicated that proteins in surimi waste water could penetrate freely through microfiltration membranes. However, the protein profiles in the retentate and permeate which are using the same membrane pore size

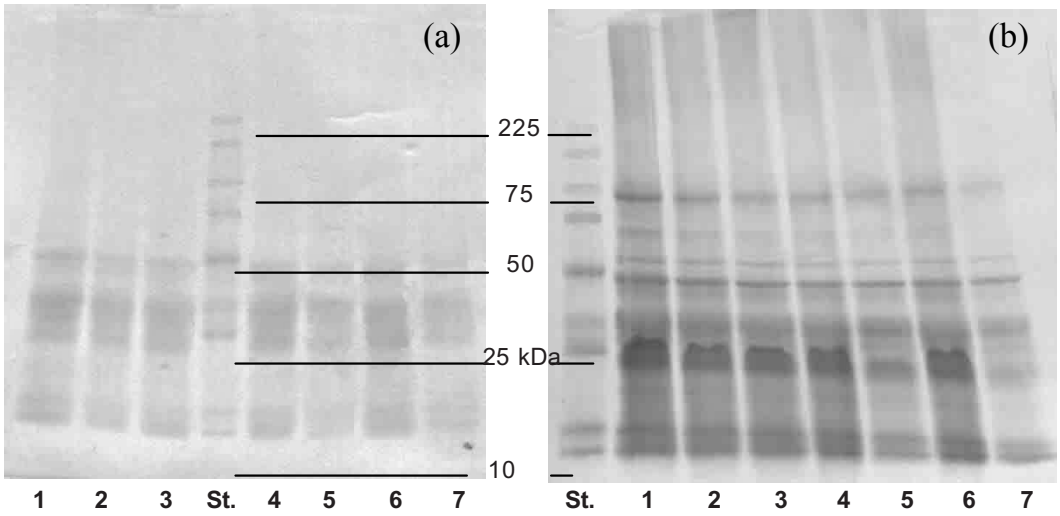

Figure 5 SDS-PAGE of surimi waste water (W1, W4) and of retentate(R) and permeate (P) of microfiltration with membrane pore size of 1, 0.45, and 0.22 μm

(a) lane 1:W1, lane2 :W1/R (1 μm), lane 3:W1/P (1 μm), lane St: standard proteins, lane 4:W1/R (0.45 μm), lane 5: W1/P(0.45 μm) lane 6:W1/R (0.22 μm) lane 7:W1/P(0.22 μm).

(b) lane St: standard proteins, lane 1: W4/R(1 μm), lane 2:W4/P(1 μm), lane 3:W4/R(0.45 μm), lane 4:W4/P(0.45 μm), 5:W4/R(0.22 μm, lane 6:W4/P(0.22 μm) and lane 7:W4.

did not differ. These results indicated that microfiltration are not suitable for fractionation of protein from surimi waste. Microfiltration with pore size of 0.05 μm has been used successfully for fractionation of milk protein [10]. Their result suggested that the difference in the molecular weigh of proteins and membrane pore size played an important role in the determination of membrane selectivity. It has been reported that pore blocking followed by cake layer formation were dominated fouling mechanisms during microfiltration of surimi washing water [1]. However, this result indicated that fouling which developed during microfiltration did not had a significant effect on membrane selectivity.

4.0 CONCLUSION

The molecular weight of proteins in surimi waste water (W1, W2, W3 and W4) used in this study was in the range of 10 to 100 kDa. Although MWCO of ultrafiltration membrane (100 and 300 kDa) used was fairly bigger than the molecular weight of protein, most proteins were retained in the retentate. The results indicated that ultrafiltration could be successfully used for recovery of these proteins but it could not be used for fractionation. This result also suggested that membrane fouling probably played an important role in the determination of membrane selectivity. Therefore, the link between the fouling and the changes in membrane selectivity during ultrafiltration

of these proteins has to be studied. In contrast, most protein could passed through microfiltration membranes (pore size : 0.22, 0.45, and 1 μm) since their size was much smaller than the membrane pore size, and the fouling layer formed during microfiltration did not have a significant effect on the protein rejection. This indicated that microfiltration could not be used to employ for fractionation of these proteins.

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