RECOVERY AND UTILIZATION OF PROTEIN DERIVED FROM SURIMI WASH-WATER

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ABSTRACT

Surimi processors are committed to improve utilization of seafood resources, increase productivity and reduce organic matter discharged into the environment. The object of this study was to recover protein from pollock surimi processing wash-water using membrane filtration and characterize properties of the recovered material. A pilot unit equipped with membrane elements concentrated protein from the surimi wash-water. Membrane concentrate and control surimi samples were analyzed for proximate composition, lipid oxidation, color, sodium dodecyl sulfate gel electrophoresis, amino acids and minerals. Membrane concentrate, membrane concentrate plus surimi and control surimi were monitored for 180 days of storage at −20°C. The membrane concentrate had significantly higher moisture and lipid, but lower protein content than surimi. As determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis, membrane concentrate proteins displayed a greater amount of lower molar mass molecules compared with surimi. The amino acid profile was comparable to control surimi and the recovered membrane concentrate proteins had similar nutritional values to that of surimi. The results indicate that the addition of 5% membrane concentrate to surimi will not adversely affect the storage at −20°C and that the recovered wash-water protein could be used to obtain a fish protein ingredient or added back at a low percentage to surimi products.

PRACTICAL APPLICATIONS

In order to increase productivity and improve utilization of seafood resources, surimi processors are looking into alternative technologies to recover proteins and other material from the wastewater. Membrane filtration is a promising option for the concentration of wastewater. This study was conducted to determine the recovery and characterize the material recovered from surimi wash-water using a commercial membrane filtration unit. It was demonstrated that the recoverable material is nutritionally similar to the final surimi product and that the overall yield can be increased using membrane technology. In addition to the benefit of recovering protein, the membrane filtration can reduce the amount of material in the waste stream.

INTRODUCTION

Surimi is basically concentrated myofibrillar protein obtained from fish flesh that has been extensively washed with cold water. Surimi is a raw food ingredient used in a variety of products that have become increasingly popular due to their unique textural properties, storage properties and high nutritional value (Akil et al. 2008; Park and Morrissey 2000; Bourtoom et al. 2009). In the industrial surimi manufacturing process, minced flesh is repeatedly washed with chilled water to remove
sarcoplasmic proteins, lipids and lower molar mass water soluble materials, leaving a tasteless and odorless myofibrillar protein product. Although this washing process effectively lowers many enzymes associated with muscle protein degradation, the protein material washed-free has a number of potential uses. Surimi wash-water typically contains 0.5–2.3% protein (Lin et al. 1995; Morrissey et al. 2000; Park and Morrissey 2000). However, new and different technologies are needed to recover these proteins economically.

In the seafood industry, solid waste from surimi processing is usually converted to fishmeal. However, liquid waste with low solids content is often discarded into the plant’s waste stream (Chang-Lee et al. 1989; Lin et al. 1995). The processing of Pacific whiting, Alaskan pollock and shrimp in Oregon, Alaska and Washington generates 20 million tons/year of processing water (Park and Morrissey 2000). Increasing concerns over the negative impact of wastewater discharge have led to research in protein recovery from surimi wash-water. An effective method to recover fish proteins from surimi wash-water would not only reduce the negative environmental impact and the cost of waste disposal, but could also lead to new ways to generate profit. Recovered protein could be returned to the process to increase final surimi yield. Figure 1 illustrates a mass balance for fish solids in a typical surimi processing facility, including an alternative membrane filtration concentration for press-water. Overall, the mass balance will vary depending on species, harvesting season and plant operations. In all cases, the loss of fish solids in wash-water during surimi washing remains significant.

There have been several studies on recovering proteins from surimi processing wash-waters. Ultrafiltration has been used to produce protein concentrates with good functional properties via myofibrillar proteins recovered from surimi wash-water (Morr 1976; Chang-Lee et al. 1989; Lin et al. 1995; Morrissey et al. 2000). Severe fouling of the membranes has been a frequent problem (Morr 1976). Huang and Morrissey (1998) evaluated the microfiltration membrane fouling by surimi wash-water and reported that fouling occurred initially as a result of pore blocking resistance followed by cake resistance. Jaouen and Quennmeneur (1992) noted that processing of surimi wash-water by ultrafiltration without pretreatment was not practical. However, Mireles Dewitt and Morrissey (2001) showed that large molar mass proteins that interfered with ultrafiltration could be removed by first adjusting wash-water acidity to pH 6 and then applying a rapid heat treatment to raise the temperature to 60°C.

Attempts to combine ohmic heat treatments with ultrafiltration on surimi wash-water were also investigated. Huang et al. (1997, 1998) studied the effect of ohmic heating (70°C) on protein coagulation in surimi wash-water and found that the wash-water-soluble protein could be removed; however, an important consideration was the possibility of retaining proteolytic activity after mild heat treatment. Some proteases tend to be stable, so there is a likelihood of recovered denatured enzymes adversely affecting the final product (Scopes 1994).

Many proteins have been employed to improve the mechanical properties of surimi gels. The most frequently used are egg white and whey protein concentrates; other sources such as leguminous extracts and porcine plasma protein have also been proposed. These proteins are added to inhibit the proteolytic degradation of fish myosin when gels are incubated at 60°C, and to favor gel setting by the action of endogenous and added transglutaminases (An et al. 1996; Garcia-Carreno 1996; Sanchez et al. 1998; Benjakul et al. 2001).

Previous studies have shown that using ultrafiltration could enable greater than 65% recovery of proteins (Afonso et al. 2004). If the recovered protein was added back to the surimi cake it would increase productivity and generate greater revenues (Afonso et al. 2004). The objectives of this project were: (1) evaluation of the potential for applying membrane filtration technology to the recovery of the protein from surimi wash-water; (2) the chemical characterization of the recovered protein; and (3) the evaluation of the properties of the concentrated recovered protein when added back to surimi prior to a 180-day frozen storage study.
**MATERIALS AND METHODS**

**Membrane Filtration Unit and Samples**

Membrane filtration was conducted on wash-water generated by the standard surimi processing technology used in Alaskan seafood processing plants. The tests were initiated immediately after collection of samples, and the filtration test was carried out maintaining the wash-water temperature (4°C).

For this study, a spiral wound 8-in. filtration module manufactured by Kelitec Engineering (Laguna Hills, CA) was used. Tests were carried out at Westward Seafood’s Unalaska, Dutch Harbor, AK plant during the Pollock (*Theragra chalcogramma*) B season utilizing the pilot plant-sized membrane filtration unit equipped with two membrane elements. The membranes had a nominal pore size of 80 kDa, and the membrane substrate was polyacrylic nitrile (PAN). This configuration provides a compact, cost-effective membrane system where high cross flow rates are required while avoiding premature protein-induced fouling.

Samples obtained from Westward Seafoods Inc. in Dutch Harbor, AK, were shipped frozen to Fairbanks, AK, for analysis. The five composite samples were (1) pollock surimi serving as a negative control; (2) pollock surimi with 5% membrane concentrate (MC) added; (3) pollock surimi with 7% sorbitol and 0.25% polyphosphate added as a cryoprotectant control; (4) pollock surimi with 7% sorbitol and 0.25% polyphosphate as cryoprotectants and 5% MC added; and (5) 100% MC. Samples were stored at −20°C until evaluated. Three subsamples were taken for each analysis.

**Proximate Composition**

Proximate composition was determined in quadruplicate for each sample. Moisture and ash content were determined using AOAC methods 952.08 and 938.08, respectively (AOAC 1980). Nitrogen content was determined by pyrolysis with a Rapid N3 (Elementar America Inc., Mt. Laurel, NJ) nitrogen analyzer. Protein content was calculated as 6.25 times %N. Total lipid content was determined gravimetrically by the Folch method (Folch et al. 1957). After lipid extraction, the tissue was dried at 4°C until eval-

**Electrophoresis**

The sodium dodecyl sulfate polyacrylamide gel electrophoresis system (SDS-PAGE) was used with a Photodyne Foto/Force 300 electrophoresis apparatus under reducing conditions according to Schagger and Von Jagow (1987). Precast 10–20% Tricine gels (Novex, Invitrogen, Carlsbad, CA) were used and molar mass standards were purchased from Sigma-Aldrich.

The protein bands were visualized from the gels stained with Coomassie blue (Sigma-Aldrich, St. Louis, MO).

**Amino Acid Analysis and Mineral Analysis**

Amino acid profiles were determined by the AAA Service Laboratory Inc., Boring, OR. Samples were hydrolyzed with 6 N HCl and 2% phenol at 110°C for 22 h. Amino acids were quantified using the Beckman 6300 analyzer with postcol-

**Lipid Oxidation**

The procedure was modified from Siu and Draper (1978). TBA reagent was prepared by adding 300 mg 2-thiobarbituric acid (TBA, MP Biomedical, Solon, OH) into 100 mL DI. Five-

**Whiteness**

Surimi color and whiteness are important factors affecting product quality and, ultimately, price. Cooked surimi samples were chopped with a spatula, placed on a Hunter Lab Color-

**Storage Study**

To examine the effects of storage, the samples were stored at −20°C and examined at days 0, 30, 90 and 180. Upon receipt of...
samples, a small amount was removed for day 0 analysis. The remaining sample was placed inside whirl pack bags and stored in a commercial freezer at -20°C until analysis.

**Statistical Analysis**

The averages and standard deviations were calculated using Excel (Microsoft). For tests of statistical difference between data sets, the data were subjected to analysis of variance followed by a post hoc Tukey’s honest significant difference test ($P < 0.05$) using Statistica version 6.0 (StatSoft Inc., Tulsa, OK).

**RESULTS AND DISCUSSION**

**Membrane Filtration**

Selection of the filter membrane molecular cutoff determines the percentage and type of product recovered as well as the filtration rate. Small-scale preliminary tests carried out at the Fishery Industry Technology Center in Kodiak, AK, found that membranes with a molecular cutoff of 50 kDa resulted in recovery of approximately 80% of the protein contained in the wash-water while salts and smaller organic molecules still passed through the membrane. Experiments indicated that membranes with a molecular cutoff between 50 and 100 kDa achieved a good balance between recovery and filtration rates (flux). Selection of the appropriate membrane material also determines the methods required to clean the membrane effectively between runs. Ceramic and polymeric PAN M series (polyacrylic nitrile) membranes were found to have superior performance under the conditions employed here.

The filtration rate obtained for two consecutive days of operation in a commercial surimi plant is shown in Fig. 2. In test 1, the initial feed volume was 2,850 L concentrated to 420 L over an 8-h period. In test 2, the initial volume was also 2,850 L, this time concentrated to approximately 200 L over a 7-h period, which resulted in a highly viscous concentrate.

The test showed that high cross flow rates (340 L/min per element) and very low transmembrane pressures (8–10 psi) were required to avoid fouling of the membrane surfaces (Fig. 2). At these settings, the membrane filters maintained reasonable flux levels during the 7–8-h test periods without showing any noticeable degradation.

The viscosity of the recovered product increased rapidly at concentrations greater than 10% solids. At 14% solids, the recovered product became very thick and difficult to pump. The upper concentration limit with the applied membrane system was approximately 12% solids. The solids content of both the feed and the concentrate is shown in Table 1.

With the 80 kDa cutoff, product recovery was approximately 75% of the solids. This resulted in the recovery of the product containing the higher molar mass molecules, while the salts and smaller organic molecules still pass through this type of membrane. The protein content in the recovered product was similar to that of surimi when adjusted for different moisture content.

**Proximate Analysis**

The initial proximate analyses of the five surimi samples are presented in Table 2. The composition of MC was very similar to previously published results for recovered protein from surimi wastewater (Lin et al. 1995). The MC had significantly
higher moisture and lipid content, and lower protein content than the surimi samples. There was no difference in the ash composition of the samples.

**Electrophoresis**

The SDS-PAGE analysis of surimi samples is presented in Fig. 3A,B. The banding pattern for the gels analyzing storage day 0 for cryoprotected surimi and cryoprotected surimi + 5% MC agrees well with previously published gels of surimi analog crabstick product prepared with Alaska pollock surimi (Reed and Park 2008). As expected, surimi samples show myosin heavy chain bands at approximately 200 kDa, actin bands at approximately 40 kDa, and myosin light chain bands located between 20 and 13 kDa, while the MC showed only trace amounts of protein at these molar masses. The abundance of low molar mass bands in the MC is in agreement with previously published results (Lin et al. 1995; Bourtoom et al. 2009). Few differences were seen between the gels loaded with samples from day 0 (Fig. 3A) and day 180 (Fig. 3B), indicating protein stability.

**Mineral Analysis and Amino Acid Analysis**

The amino acid profile of normal surimi (1a) and MC (3) is presented in Table 3. Values for three of the basic amino acids were 9.0% for lysine, 6.1% for arginine and 2.9% for histidine. Methionine (3.5%) and phenylalanine (6.8%) concentrations in MC were higher than in previously published fish and soy meal analyses (Ohshima et al. 1993; Wibowo et al. 2005). These results indicate the MC, in terms of amino acid composition, has values comparable with surimi. The percentage of total essential amino acids and total nonessential amino acids in MC was 45.0 and 55.0%, respectively.
The mineral composition of normal surimi, cryoprotected surimi and MC is presented in Table 4. The mineral concentrations were similar for surimi and MC; however, higher levels of Fe, Zn and Cu were present in MC.

Whiteness and Lipid Oxidation

Whiteness data are reported at storage time points over the 180 days in Fig. 4. The color of the MC appeared slightly darker than the other surimi samples for all storage with values ranging from 55 to 60. The darker color was also consistent with the higher Fe values for MC. Whiteness in surimi and surimi with added MC did not decrease dramatically over the 180 days of storage ($P < 0.05$).

The extent of lipid oxidation over the storage time is shown in Fig. 5. At day 180, the TBA values in all samples are higher than those found in all previous samplings. Also, the MC has a higher amount of MDA present in all samples compared with the other treatments. This can be explained by the higher lipid content in the MC and possibly the increased Fe in this fraction. The results indicate that lipid oxidation increases with time and samples with higher lipid levels show greater lipid oxidation. TBA values did increase in most treatments with storage time although generally not significant. There was a trend for higher TBA values in surimi with added MC than samples where MC was absent.

### Table 3. Comparison of Amino Acid Profile of Pollock Surimi and Membrane Concentrate

<table>
<thead>
<tr>
<th></th>
<th>Surimi</th>
<th>Surimi + 5% MC</th>
<th>MC</th>
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<tr>
<td></td>
<td>Ave.</td>
<td>Ave.</td>
<td>Ave.</td>
</tr>
<tr>
<td>ALA</td>
<td>5.8</td>
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<td>6.1</td>
</tr>
<tr>
<td>ARG</td>
<td>7.7</td>
<td>6.1</td>
<td>6.1</td>
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<tr>
<td>ASP</td>
<td>11.1</td>
<td>12.6</td>
<td>12.6</td>
</tr>
<tr>
<td>GLU</td>
<td>15.4</td>
<td>11.2</td>
<td>11.2</td>
</tr>
<tr>
<td>GLY</td>
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<td>4.45</td>
<td>4.45</td>
</tr>
<tr>
<td>HIS</td>
<td>2.4</td>
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<td>2.9</td>
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<tr>
<td>ILE</td>
<td>5.6</td>
<td>5.6</td>
<td>5.6</td>
</tr>
<tr>
<td>LEU</td>
<td>9.3</td>
<td>9.2</td>
<td>9.2</td>
</tr>
<tr>
<td>LYS</td>
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<td>9.1</td>
<td>9.1</td>
</tr>
<tr>
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<td>3.5</td>
<td>3.5</td>
</tr>
<tr>
<td>PHE</td>
<td>4.3</td>
<td>6.8</td>
<td>6.8</td>
</tr>
<tr>
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<td>3.5</td>
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<td>4.0</td>
<td>4.0</td>
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<td>THR</td>
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<td>4.5</td>
<td>4.5</td>
</tr>
<tr>
<td>TYR</td>
<td>4.6</td>
<td>3.9</td>
<td>3.9</td>
</tr>
<tr>
<td>VAL</td>
<td>5.7</td>
<td>6.5</td>
<td>6.5</td>
</tr>
<tr>
<td>TEAA %</td>
<td>43.7</td>
<td>45.0</td>
<td></td>
</tr>
<tr>
<td>TNEAA %</td>
<td>56.2</td>
<td>55.0</td>
<td></td>
</tr>
</tbody>
</table>

Wat/wat % total amino acids measured. TEAA is total essential amino acids. TNEAA is total nonessential amino acids.

MC, membrane concentrate; Ave., average; SD, standard deviation.

### Table 4. Mineral Composition of Surimi Samples

<table>
<thead>
<tr>
<th>Minerals</th>
<th>Surimi</th>
<th>Surimi + Cryo + 5% MC</th>
<th>MC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ave.</td>
<td>Ave.</td>
<td>Ave.</td>
</tr>
<tr>
<td>P (%)</td>
<td>0.26a</td>
<td>0.36a</td>
<td>0.70a</td>
</tr>
<tr>
<td>K (%)</td>
<td>0.23a</td>
<td>0.18a</td>
<td>0.39a</td>
</tr>
<tr>
<td>Ca (%)</td>
<td>0.15a</td>
<td>0.12a</td>
<td>0.22a</td>
</tr>
<tr>
<td>Mg (%)</td>
<td>0.14a</td>
<td>0.11a</td>
<td>0.17a</td>
</tr>
<tr>
<td>Na (ppm)</td>
<td>2,724a</td>
<td>3,473b</td>
<td>3,791b</td>
</tr>
<tr>
<td>Cu (ppm)</td>
<td>0.95a</td>
<td>0.56a</td>
<td>4.35b</td>
</tr>
<tr>
<td>Zn (ppm)</td>
<td>19a</td>
<td>14b</td>
<td>39.33b</td>
</tr>
<tr>
<td>Mn (ppm)</td>
<td>1.33</td>
<td>ND</td>
<td>1</td>
</tr>
<tr>
<td>Fe (ppm)</td>
<td>8a</td>
<td>5.66</td>
<td>47.33b</td>
</tr>
</tbody>
</table>

Different alphabetical letters indicate a significant difference ($P < 0.05$) within each row.

MC, membrane concentrate; SD, standard deviation of the mean; ND, below detection limit.
Storage Study

There were no dramatic changes in proximate composition, minerals, amino acids or whiteness over the 180 days of the study. There was however noticeable increase in lipid oxidation at day 180 as determined by TBA values, especially in the MC. This is consistent with previous results demonstrating that higher lipid values result in increased oxidation (Matsushita et al. 2010). However, the results indicate that the addition of MC to surimi will not adversely affect the storage at −20°C.

CONCLUSIONS

Solids from surimi wash-water were successfully recovered using an ultrafiltration system. Protein concentrates recovered in these experiments had a significantly higher moisture and lipid content when compared with surimi. The proteins concentrated by membrane filtration displayed a larger number of lower molar mass compared with surimi. The amino acid profiles of the MC were comparable to that of surimi. The results indicate that the addition of 5% MC to surimi will not adversely affect the storage at −20°C. From the results of this study, it is possible that the recovered wash-water protein could be used to obtain a fish protein ingredient or added back at a low percentage to surimi products.

REFERENCES


