

Potentiality of Proteins Recovery from Surimi Washwater using UF Crossflow System

(Potensi Pemulihan Protein daripada Air Basuhan Surimi menggunakan Sistem Aliran Silang UF)

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ABSTRACT

Washwater from surimi production contains significant amounts of proteins and other useful components, which are typically discharged directly without recovery procedures. Additionally, the washing phase in surimi processing consumes a significant quantity of water, and the direct discharge from surimi production adversely impacts the environment. The ultrafiltration (UF) process not only extracts proteins that otherwise complicate washwater treatment but also enables the reuse of large volumes of water. This study aimed to assess the efficacy of UF in the fractionation of proteins from surimi washwater. A PVDF membrane with a molecular weight cut-off (MWCO) of 100 kDa was used to separate these proteins using a bench-scale crossflow filtration system setup. This investigation evaluated several varieties of local, white-fleshed fish and freshwater fish as samples, namely Shark Catfishes (F1), Threadfin Bream (F2), Red Tilapia (F3), Obtuse Barracuda (F4), and Peters' Monocle Bream (F5). F5 exhibited the highest total protein concentration of 2346.25 mg/mL in comparison to the other types of fish. The flux marked a range of rejection of 21%-63% of total protein content. Further investigation was carried out using Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) to segregate proteins based on their electrophoretic mobility. In SDS-PAGE analysis, each type of fish exhibited distinct band patterns according to the various proteins present in the fish, and eventually UF presented a promising approach for the protein recovery process for a broad spectrum of protein based on MWCO.

Keywords: Crossflow filtration; protein recovery; surimi washwater; ultrafiltration membrane; washwater treatment

ABSTRAK

Air basuhan daripada penghasilan surimi mengandungi sejumlah besar protein dan komponen bermanfaat lain, yang kebiasaannya dilepaskan terus tanpa sebarang prosedur pemulihan. Selain itu, fasa pencucian dalam pemprosesan surimi menggunakan kuantiti air yang banyak dan pelepasan terus daripada pengeluaran surimi memberi kesan negatif terhadap alam sekitar. Proses ultraturasan (UF) bukan sahaja mengekstrak protein yang sebaliknya menyukarkan rawatan air basuhan, malah membolehkan penggunaan semula jumlah air yang banyak. Seterusnya, pengabluran pukal boleh digunakan untuk memurnikan sebahagian protein yang diperolehi, sekali gus menambah nilai kepada pendekatan cekap sumber ini. Kajian ini bertujuan menilai keberkesanan UF dalam pemecahan protein daripada air basuhan surimi. Membran PVDF dengan had potongan berat molekul (MWCO) 100 kDa telah digunakan untuk memisahkan protein ini melalui sistem penurasan aliran silang berskala makmal. Kajian ini menggunakan beberapa jenis ikan berdaging putih tempatan dan ikan air tawar sebagai sampel, iaitu ikan Patin (F1), Kerisi Bali (F2), Tilapia Merah (F3), Kacang (F4) dan Timun (F5). F5 menunjukkan kepekatan protein keseluruhan tertinggi iaitu 2346.25 mg/mL berbanding jenis ikan lain. Fluks mencatatkan julat penolakan sebanyak 21%-63% daripada jumlah kandungan protein bagi kesemua

jenis ikan. Penyelidikan lanjut dijalankan menggunakan Sulfat Natrium Dodekil Gel Elektrophoresis Poliakrilamida (SDS-PAGE) untuk mengasingkan protein berdasarkan mobiliti elektroforetiknya. Dalam analisis SDS-PAGE, setiap jenis ikan menunjukkan corak jalur yang berbeza mengikut pelbagai protein yang terdapat pada ikan tersebut dan akhirnya UF menunjukkan pendekatan yang berpotensi untuk proses pemulihan protein bagi spektrum luas protein berdasarkan MWCO.

Kata kunci: Air basuhan surimi; membran ultraturasan; pemulihan protein; penurasan aliran silang; rawatan air basuhan

INTRODUCTION

Surimi is a type of extensively processed seafood derived from minced fish (Luo et al. 2022). Demersal fish, characterised by white flesh and bottom-dwelling habits, are the most appropriate raw material for surimi manufacturing compared to pelagic or dark-fleshed fish, as they contain more protein and will produce a high yield of surimi (Yingchutrakul et al. 2022). Currently, the global market for surimi stands at 500,000 tonnes, with India contributing approximately 45,000 tonnes to this total amount (Shamasundar 2023). Southeast Asian countries, including Thailand, Malaysia, Vietnam, Myanmar, and Indonesia, mostly produce surimi from demersal species such as Threadfin Bream (*Nemipterus* sp.), Big Eye (*Priacanthus* sp.), and Lizardfish (*Saurida* sp.), as these species are the most abundant raw materials in the region and possess the requisite characteristics for processing export-quality surimi (Sihono et al. 2021).

In the surimi manufacturing industry, washing is a crucial step during which lipids, water-soluble proteins, pigments, and other elements that hinder gel formation are removed from the fish (Song et al. 2024). It is worth mentioning that the substantial quantity of carbon-based materials has been highlighted as an underexploited resource with immense potential for energy, fuel, and organic recovery (Das, Verma & Mishra 2023; Hou et al. 2020; Pham et al. 2015). The fish processing sector produces a substantial array of by-products and waste, amounting to nearly 57% (w/w) of the total weight of captured fish, which are often categorised as follows: 9-12% (heads), 12-18% (viscera), 1-3% (skin and fins), 15-20% (filleting waste), 9-15% (bones), and 5% (abdominal contents) (Rustad, Storror & Slizyte 2011). Notwithstanding their abundant nutritional worth, majority of them are disposed of in landfills or water bodies without prior nutrient recovery (Lee et al. 2016), resulting in the squandering of valuable nutrients, significant environmental degradation, and increased operational expenses (Baraiya et al. 2023). Consequently, washing generates large volumes of washwater that could negatively affect the natural environment if released untreated.

Additionally, processing of surimi and other seafood industries will produce waste, either solid or liquid. Solid waste can usually be used as animal feed or fishmeal, whereas liquid waste is often directly discharged

without any treatment (Ghalamara et al. 2024), causing detrimental effects on the environment. Furthermore, the primary concern is that proteins present in washwater are discharged directly into the stream without any recovery or removal step, resulting in the waste of valuable resources such as beneficial proteins contained in surimi washwater. Statistically, about 30 L of washwater is discharged to produce 1 kg of surimi (Lin, Park & Morrissey 1995). Upon the release of industrial effluents into the environment, they integrate into the air, soil, surface water, and groundwater, subject to the nature of the pollutants discharged (Iftikhar et al. 2023).

Sustainable waste management and resource reclamation options become pertinent considering the rising concern over untreated organic content effluents. The washwater generated in the fishmeal industry, which is water-intensive, contains a high organic load with a significant number of proteins. This recognition has been strengthened by numerous studies investigating the use of organic compounds derived from food and plant waste (Sahari et al. 2023). Hence, these proteins have high commercial value if they can be separated from the sludge. Proteins found in surimi washwater can be efficiently recovered and concentrated through ultrafiltration (UF) membrane technology by transforming a by-product stream into a sustainable source of high-quality protein.

Significantly, one of the most viable methods for using these processing byproducts is to extract nutrients for human use (Baraiya et al. 2023). Numerous efforts have been reported to repurpose fish waste into valuable products, as it has potential as an excellent source of human nutrition and is rich in proteins and fats. The nutrients can be categorised for human consumption (bioactive peptides, lipids, chitin, chitosan, roe, gelatine, and nutraceuticals), agricultural or related purposes (fertilisers and compost), non-nutritional uses (biodiesel and fuel, carotenoids, pigments, leather), cosmetics (collagen), and enzyme isolation (Sahana et al. 2025). Notably, this sector produces significant solid and liquid by-products, with variations in composition based on species and processing techniques (Muhamad Nor Iqmal et al. 2025). One particular concern is that the protein extraction potential of fish by-products is substantially influenced by the significant variation in protein content among fish species, which is characterised by a wide range of protein molecular weights.

Therefore, proper treatment of surimi washwater plays a crucial role in mitigating environmental impact and ensuring resource conservation. This study investigates the potential of utilising UF membranes to attain simultaneous achievement of both treated washwater from surimi production and high protein recovery. Despite its significant advantages, a better understanding of the protein composition across different fish species is crucial prior to the applicability of the ultrafiltration process for protein separation, ensuring its application is optimized and fully utilized. Additionally, information on protein rejection reflects the filtration performance of the UF membrane. To date, the various types of white-fleshed fish used in this study have not been extensively investigated. Moreover, there is a lack of comprehensive literature comparing the protein content among these different fish species. The outcome of this study will be of significant interest and a valuable reference for research on water treatment in surimi production applications and protein recovery areas, especially concerning membrane technology.

MATERIALS AND METHODS

The outline of the methodological approach is briefly described in Figure 1. The succeeding sections provide an extensive explanation of the materials and methods involved.

PREPARATION OF SURIMI WASHWATER SAMPLE

Five local fish species obtained from a local fish market were chosen as the raw materials for the analysis, namely Shark Catfishes (*Pangasiidae* sp.), Threadfin Bream (*Nemipterus* sp.), Red Tilapia (*Oreochromis* sp.), Obtuse Barracuda (*Sphyraena obtusata*), and Peters' Monocle Bream (*Scolopsis affinis*). These white-fleshed fish were designated as F1, F2, F3, F4, and F5, respectively. Fish mince was produced by deboning at a low temperature to protect the sample from frictional

heat. The minced fish was then washed with cold water at a water-to-mince ratio of 3:1 to obtain the surimi washwater. Then, salt (0.2% of the mince weight) was added to the surimi washwater to remove blood and loose scales. Salt also promotes protein aggregation by hydrophobic surfaces. The mixture was then centrifuged at 10,000 g for 25 min. The supernatant was collected and used as model surimi washwater loaded in the system as feed.

DETERMINATION OF TOTAL PROTEIN CONTENT

Total protein content was measured by using a UV-visible spectrophotometer. Protein content was determined according to the calibration curve by using bovine serum albumin (BSA; Sigma) as the protein standard (Bradford 1976). The wavelength used to measure the spectrophotometer absorbance was 595 nm.

PROTEIN ASSAY OF SURIMI WASHWATER

Protein assay was performed to compare protease activity between the retentate and permeate samples. Both acidic and alkaline protease activities were considered in this assay by using haemoglobin as an inhibitor. The samples were mixed with the inhibitor at a ratio of 1:9 (0.1 mL sample: 0.9 mL inhibitor). Then, these mixtures were incubated in a 37 °C water bath for 30 min. After that, 0.4 mL of 20% w/v Trichloroacetic acid (TCA) was added to the mixtures and centrifuged at 4000 rpm for 15 min at 4 °C to stop the protease activity. Finally, the absorbance reading of the supernatant was taken at 410 nm for alkaline protease and at 280 nm for acidic protease. Specific activities of acidic and alkaline proteases were calculated as follows;

$$\text{Specific activity} \left(\frac{\text{Units}}{\text{mg}} \right) = \frac{\text{Absorbance (Units)}}{\text{concentration of protein} \left(\frac{\text{mg}}{\text{mL}} \right) \times \text{volume of sample (mL)}}$$

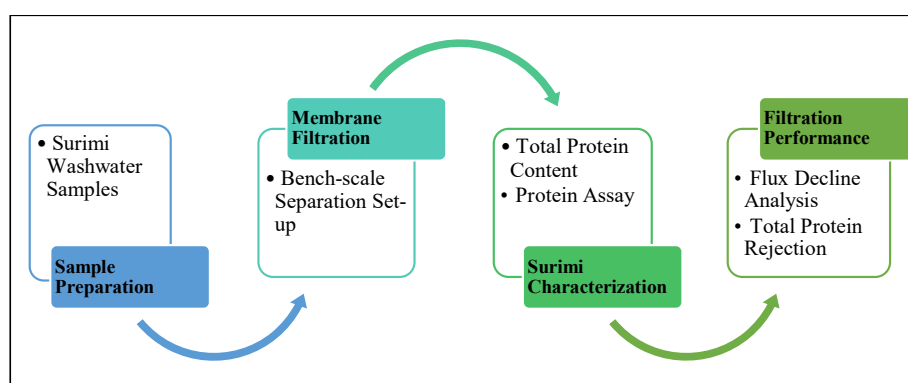


FIGURE 1. Methodological outline of the study from sample preparation to measuring filtration performance

SODIUM DODECYL SULPHATE-POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE) ANALYSIS

SDS-PAGE analysis was carried out to determine the components of a protein mixture. Two mini polyacrylamide gels with different concentrations (12% and 5%) were used for the SDS-PAGE. The solutions that are used to prepare the resolving gel for Tris-glycine SDS-PAGE are deionized water (dH₂O), 30% Acrylamide mix, 1.5 M Tris (pH 8.8), 10% SDS, and TEMED (Tetramethylethylenediamine).

6 µL of retentate and permeate collected from the ultrafiltration step were mixed with 3 µL of Laemmli buffer (Bio-Rad) and centrifuged for 2 min. The mixtures were then heated to 95 °C for 5 min to denature the protein. 8 µL of the heated mixtures and 5 µL of molecular weight markers (Table 1) were loaded into the polyacrylamide gel. Gels were run at a constant voltage of 120 V for 1 h. Gels were then stained in 0.125% Coomassie Brilliant Blue R-250 and gently shaken for about 45 min to 1 h. Gels were destained with 25% ethanol and 10% acetic acid solution until the blue colour faded for better visualization.

ULTRAFILTRATION PROCESS USING A CROSS-FLOW SYSTEM

This study uses a cross-flow UF system at low-pressure operation of transmembrane pressure (TMP) 1.0 bar, which has a filtration surface that is continuously swept by flowing liquid. The filtration set-up and schematic diagram were explained in detail in previous studies (Ali, Kamarudin & Ismail 2017; Kamarudin et al. 2015). Commercial tubular membranes acquired from PCI Membranes, Poland, made from polyvinylidene fluoride (PVDF) with a molecular weight cut-off (MWCO) of 100 kDa, a fibre diameter of 63.5 mm, a flow length of 30 cm, and an effective area of 0.024 m², were used in this study. The crossflow pressure was established at an operating pressure of 0.3 bar, with the temperature regulated at 40 °C, and the feed volume maintained at 6 L.

During the filtration process, the feed solution was initially placed in the feed tank and continuously circulated through the membrane module using a pump. The feed stream was separated into two streams: the permeate, which passed through the membrane, and the retentate, which was retained by the membrane. The retentate stream was continuously returned to the feed tank, allowing continuous recirculation and mixing of the retained fraction with the bulk feed solution. The permeate was collected separately for subsequent analysis.

Samples were collected from three designated points in the filtration system, namely the feed, permeate, and retentate streams. The feed sample was collected directly from the feed tank prior to the filtration process. The permeate sample was collected from the permeate outlet after passing through the membrane. The retentate sample was collected from the retentate outlet immediately before the retentate stream was returned to the feed tank. Therefore, the retentate concentration reported in this study represents the concentration of the recirculating retentate stream at the sampling point and does not necessarily represent a concentrated end-product obtained after batch concentration. All collected samples were subjected to further protein concentration and protease activity analyses.

Before the ultrafiltration step, the pure water flux of the membrane was determined and used further to calculate the normalised flux of surimi washwater. Protein concentration of feed (C_f), permeate (C_p), and retentate (C_r) were determined during the UF process. The percentage of solute rejection and transmission was used to measure membrane performance and selectivity.

$$Flux = \frac{V}{t \times A}$$

where *v*, *t*, and *A*, respectively, represent permeate volume, time and surface area of the membrane.

TABLE 1. Types, molecular weight, and sources of protein in prestained SDS-PAGE Standards (broad range)

Types of protein	Molecular weight (kDa)	Sources
Myosin	200	Rabbit skeletal muscle
β-Galactosidase	116	<i>E. coli</i>
Bovine Serum Albumin (BSA)	66	Bovine plasma
Ovalbumin	45	Chicken egg white
Carbonic anhydrase	30	Bovine erythrocytes
Protease	25.8	Surimi washwater
Soybean trypsin inhibitor	20.1	Soybean
Lysozyme	14.3	Chicken egg white
Aprotinin	6.5	Bovine pancreas

The percentage of rejection was calculated using the formula below;

$$\text{Percentage of rejection, } R (\%) = \left[1 - \left(\frac{C_P}{C_F} \right) \right] \times 100\%$$

$$\text{Percentage of transmission, } T (\%) = 100\% - R$$

RESULTS AND DISCUSSION

CHARACTERISATION OF SURIMI WASHWATER SAMPLE

There are five types of local white-fleshed fish, which include Shark Catfishes (F1), Threadfin Bream (F2), Red Tilapia (F3), Obtuse Barracuda (F4), and Peters' Monocle Bream (F5). Representative images of the fish are shown in Figure 2. Samples of surimi washwater from five distinct fish species were separately characterised for total protein content, proteolytic activity, and molecular weight. These analyses were carried out before and after the ultrafiltration procedure. This investigation aimed to assess the degree of protein recovery and to ascertain if proteolytic enzymes in the washwater facilitated protein degradation during processing. Evaluating both parameters offered crucial information on the efficacy of ultrafiltration in separating intact proteins while also showing potential losses or quality alterations resulting from enzymatic hydrolysis, thus

facilitating a more precise assessment of the recovery potential of the washwater proteins.

PROTEOLYTIC ACTIVITY

The analysis of proteolytic activity in conjunction with ultrafiltration ensures that the recovered proteins are not substantially degraded by proteases, evaluates the suitability of the membrane process, and ultimately ascertains whether the recovered proteins are appropriate for recovery or further processing techniques. The proteolytic activity for all samples was tabulated as in Table 2.

Based on the analysis, under acidic condition, F2 exhibited the highest protease activity in the feed tank which was $2.88 \text{ E}^{-04} \text{ unit/mg}$, while F1 showed the highest activity in both retentate and permeate streams ($2.57 \text{ E}^{-04} \text{ unit/mg}$ and $7.41 \text{ E}^{-04} \text{ unit/mg}$, respectively). Similarly, for the alkaline test, F2 showed the highest reading for the feed tank compared to others, which was at $4.13 \text{ E}^{-04} \text{ unit/mg}$. Also for F2, the value for the retentate stream is $5.14 \text{ E}^{-04} \text{ unit/mg}$ and for the permeate stream was $6.28 \text{ E}^{-04} \text{ unit/mg}$.

The low solubility of proteins in alkaline-acid-assisted processes is likely attributed to changes in pH, which induce denaturation of muscle proteins. Such denaturation reduces protein solubility and enhances hydrophobic interactions, ultimately leading to protein precipitation (Zayas 1997). Kristinsson and Hultin



FIGURE 2. Images of 5 different types of fish used in this study namely (a) Shark Catfishes - F1, (b) Threadfin Bream - F2, (c) Red Tilapia - F3, (d) Obtuse Barracuda - F4, and (e) Peters' Monocle Bream - F5

(2004) reported that improper unfolding of haemoglobin reduced its solubility. Comparisons of protein isolates here showed that the alkaline-aided process resulted in greater protein solubility than the acid-aided process. Similarly, Zayas (1997) observed that both protein solubility and extraction yield were higher at alkaline pH compared to acidic conditions.

The extractability of proteases from F3 muscle was evaluated using conventional washing, acid-aided, and alkaline-aided processes. Protein recovered through the acid- and alkaline-aided methods contained higher levels of proteases compared to the conventional surimi washing. This increased extractability may be attributed to greater degradation of muscle proteins, which enhances the efficiency of proteases removal from disrupted muscle tissue (Batista, Pires & Nelhas 2007; Rawdkuen et al. 2009). Consistent with this, Chaijan et al. (2006) reported that alkaline solubilization was particularly effective in eliminating myoglobin from sardine and mackerel muscles. Nevertheless, the efficiency of myoglobin extraction is influenced by several factors, including fish species, muscle type, storage duration, and the washing method employed.

PROTEIN MOLECULAR WEIGHT DETERMINATION VIA GEL ELECTROPHORESIS

Gel electrophoretic analysis was conducted to assess and confirm the purification results. Protein fractionation was conducted on only three (F1, F2 and F3) of the five initial samples, as the analysis was intended to provide initial information on the diversity of protein species; the chosen samples represented the highest and lowest total protein contents to encompass the extensive contrast among the fish examined. The molecular weight marker that is used is Prestained SDS-PAGE Standards (Broad Range). This marker represents eight types of protein molecular weight, such as Myosin, β -Galactosidase, BSA, Ovalbumin, Carbonic anhydrase, Soybean trypsin inhibitor, Lysozyme, and Aprotinin (Figure 3). The range of molecular weight ranges from 200 to 6.5 kDa. Protease, which is 28.5 kDa, is included in this range.

Two sets of gels were used to further investigate the fractionation of protein from F1, F2 and F3. Figure 4(a) shows the first gel denoted as G1 and Figure 4(b) shows the second gel denoted as G2. G1 consists of 10 lanes, which contain 9 lanes of samples and 1 lane for the standard. Lane 1 is a molecular weight marker (ladder), lanes 2 to 7 are for F2 samples, which contain the raw sample (lane 2), initial feed (lane 3), final feed (lane 4), initial retentate (lane 5), final retentate (lane 6), and one-hour permeate sample (lane 7). The other remaining three lanes are for raw samples of F1 (lane 8), followed by initial feed (lane 9) and final feed (lane 10) of the F1 sample. For lanes 2 and 8, almost all bands can be seen clearly. For lanes 3, 4, 5, 6, 7, 9, and 10, the clearest

band was in the range of 66 kDa to 45 kDa. These bands appear different for the sample, as it depends on the molecular weight. The lightest protein was transmitted very fast to the bottom of the gel, while the heavy protein with high molecular weight moved slowly as it was retained at the top of the gel.

The same situation was also observed in G2, where almost all samples showed very clear visualisation in the range of 66 kDa to 45 kDa. G2 also has 10 lanes, of which the first lane is for the ladder. Lanes 2 and 3 contain the initial and final retentate for the F1 sample, respectively. Lane 4 contains the F1 permeate for 1 h. Lane 5 contains the raw sample of F3, whereas lanes 6 and 7 contain the initial and final feed of F3, respectively. The initial and final retentate for F3 are in lanes 8 and 9, while lane 10 represents the permeate of F3. This analysis implies that both the retentate and permeate streams may contain various types of proteins of multiple sizes. This is owing to the dimensions of the protein itself. Proteins over 100 kDa will be kept in the retentate, while those smaller will permeate through the membrane.

EVALUATION OF MEMBRANE ULTRAFILTRATION PERFORMANCE

This study employed a membrane separation test system to evaluate the performance of the membrane UF process. In the setup, the retentate was looped back to the feed tank, whereas the permeate was collected and measured every 5 min using a measuring cylinder. The TMP and crossflow velocity (CFV) were controlled at a constant value. The value of TMP is 0.3 bar, and the value of CFV is 3.79 L/min (1 GPM). The overall permeate fluxes of 60 min of operation were plotted as in Figure 6.

REJECTION AND TRANSMISSION OF PROTEIN

The samples of feed, retentate, and permeate were collected to calculate the value of total protein. C_F value represents the total protein in the feed tank, C_R in the retentate, and C_p in the permeate. Based on Table 3, analysis of the surimi washwater filtration performance showed distinct trends in protein concentration behaviour across several process streams. The data showed a uniform concentration gradient among all fish species, with a sequence of concentrations that can be simplified as $C_F > C_R > C_p$ except for F1. This pattern aligns well with other studies published (de Oliveira et al. 2021; Khatprathum, Siri Wongpaisaan & Youravong 2010), demonstrating the efficacy of the ultrafiltration process in partially retaining high molecular weight proteins in the retentate stream while permitting water and smaller proteins, including molecules, to pass through to the permeate side. Upon analysing the concentration factor's performance, notable

differences emerge across several fish species. F3 had the most remarkable performance with a concentration factor of roughly 2.74 (Cr/Cf ratio), signifying exceptional protein retention and concentration capacity.

F2 reached a considerable concentration with a ratio of around 1.22, whereas F1, F4, and F5 exhibited comparable but lower concentration factors, ranging from 1.07 to 1.08. The variations indicated that the efficiency of the ultrafiltration procedure significantly varied based on specific features of the fish species before the washwater preparation. The investigation of

membrane selectivity uncovered intriguing trends in protein passage to the permeate stream. F3 demonstrates the lowest permeate protein concentration of 847.50 mg/mL, indicating minimal protein loss and superior membrane selectivity. In contrast, F1 demonstrated the highest permeate concentration at 1273.75 mg/mL, indicating fundamentally different protein characteristics influenced by lower molecular weight. F4 and F5 have moderate permeate concentrations, while F2 and F3 have relatively low concentrations.

TABLE 2. Proteolytic activity of surimi washwater sample at acidic and alkaline conditions

Types of Fish	Acidic			Alkaline		
	Feed (units/mg)	Retentate (units /mg)	Permeate (units /mg)	Feed (units /mg)	Retentate (units /mg)	Permeate (units /mg)
F1	1.19 E ⁻⁰⁴	2.57 E ⁻⁰⁴	7.41 E ⁻⁰⁴	2.23 E ⁻⁰⁴	3.30 E ⁻⁰⁴	2.59 E ⁻⁰⁴
F2	2.88 E ⁻⁰⁴	1.87 E ⁻⁰⁴	8.91 E ⁻⁰⁵	4.13 E ⁻⁰⁴	5.14 E ⁻⁰⁴	6.28 E ⁻⁰⁴
F3	1.16 E ⁻⁰⁴	5.78 E ⁻⁰⁵	7.08 E ⁻⁰⁵	3.47 E ⁻⁰⁴	3.05 E ⁻⁰⁴	6.53 E ⁻⁰⁴
F4	1.22 E ⁻⁰⁴	2.47 E ⁻⁰⁴	8.63 E ⁻⁰⁵	2.29 E ⁻⁰⁴	1.90 E ⁻⁰⁴	3.16 E ⁻⁰⁴
F5	1.36 E ⁻⁰⁴	1.22 E ⁻⁰⁴	1.50 E ⁻⁰⁴	9.80 E ⁻⁰⁵	1.60 E ⁻⁰⁴	3.16 E ⁻⁰⁴

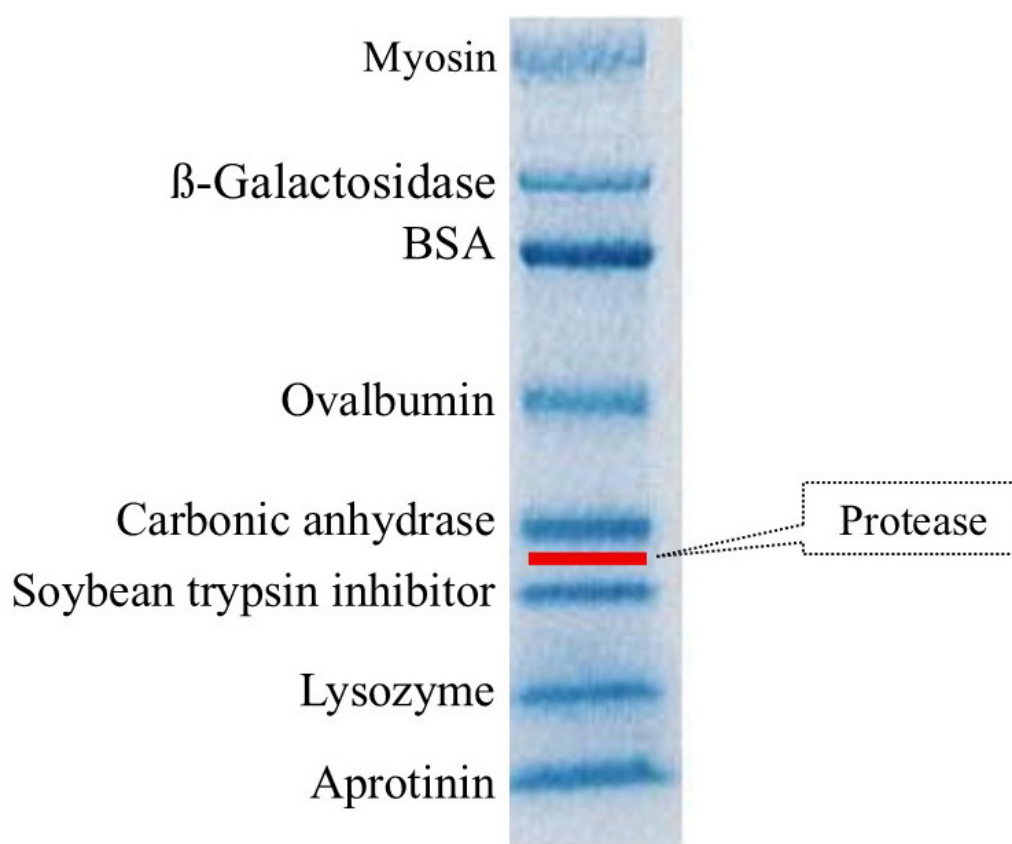


FIGURE 3. Fractionation of protein in pre-stained SDS-PAGE Standards (broad range)

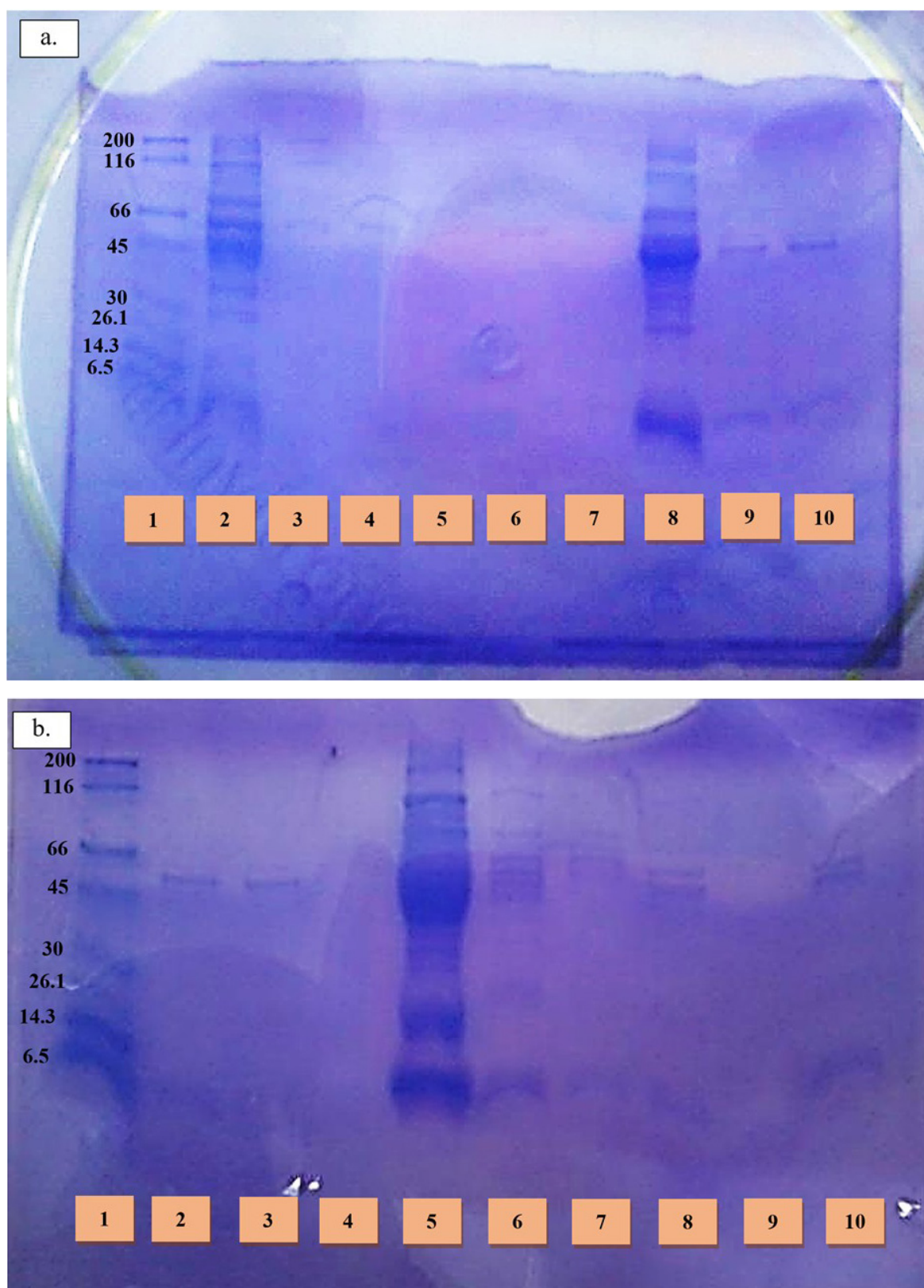


FIGURE 4. The first (a) and second (b) gel of SDS-PAGE analysis. (a) First gel (G1)—Lane 1: molecular weight marker; Lanes 2-7 (F2 samples): raw sample (2), initial feed (3), final feed (4), initial retentate (5), final retentate (6), and 1 h permeate (7); Lanes 8-10 (F1 samples): raw sample (8), initial feed (9), and final feed (10). (b) Second gel (G2)—Lane 1: molecular weight marker; Lanes 2-4 (F1 samples): initial retentate (2), final retentate (3), and 1 h permeate (4); Lanes 5-10 (F3 samples): raw sample (5), initial feed (6), final feed (7), initial retentate (8), final retentate (9), and 1 h permeate (10)

F3 exhibits optimal ultrafiltration performance, typified by high protein retention and reduced permeate losses, making it the most suitable option for efficient protein recovery procedures. In brief, the rejection and transmission rates of the UF process are shown in Figure 5. The orange colour denotes the rejection percentage, whereas the blue colour indicates the transmission percentage, as illustrated herewith. The results indicated that nearly all fish species exhibit high transmission, with the exception of F3. It may possibly be attributed to differences in the composition of its surimi washwater. Variations in the protein-to-lipid ratio and protein characteristics among fish species (Ahmed et al. 2022) may affect the solubility and molecular-weight distribution of proteins in the washwater, consequently influencing their passagethrough the ultrafiltration membrane. Other components present in the washwater may also affect membrane permeability and protein-membrane interactions. However, further characterization of the washwater composition and protein molecular-weight distribution is required to confirm the underlying mechanism.

Generally, all fish underwent a minimum of 20% rejection of total protein. The 100 kDa membrane permitted approximately 50% of the protein content to traverse the membrane. Membranes with smaller pore sizes may be more susceptible to protein fouling. The varied performance among different fish species likely reflects several fundamental factors, including differences in protein molecular weight distributions, viscosity effects on mass transfer rates, and distinct protein-membrane interaction characteristics. To better grasp the potential of the UF process, the permeation flux pattern of surimi washwater filtration for each type of fish was recorded for 60 min and discussed in the following subsection.

The transmission and rejection values were calculated based on the measured protein concentrations in the feed, permeate, and retentate streams. However, the observed decline in permeate flux suggests that concentration polarization and membrane fouling may have occurred during filtration. Protein adsorption or accumulation on the membrane surface may therefore have resulted in unquantified protein losses that were not captured by the measured permeate and retentate concentrations. Hence, the calculated transmission and rejection values should be considered apparent values based on the sampled streams rather than a complete protein mass balance. The potential contribution of such retained materials as foulants can be further evaluated using approaches reported in our previous study (Kamarudin et al. 2022).

FLUX DECLINATION PATTERN

The permeate flux profile of the 100 kDa membrane was assessed using a bench-scale filtration system with

a maximum feed volume of 10 L. The comprehensive flux decline analysis was conducted over a duration of 60 min, with permeate volumes taken at 5-min intervals. The flux declination pattern is essential for illustrating filtration performance and occurrences of fouling. Overall, throughout the filtration process, the flux experienced a rapid reduction within the first 10 min, followed by a gradual decrease until it stabilised on a consistent plateau over the subsequent 30 min. A slight decrease in flux was detected towards the end of the measurement. The results are depicted in Figure 6.

The rapid decline in flux observed during the initial few minutes was primarily attributed to concentration polarization. Subsequently, the gradual reduction in flux was likely caused by protein adsorption and the deposition of particles on the membrane surface as well as within the membrane pore walls (Tanudjaja et al. 2022). According to Marshall, Munro and Trägårdh (1993), several factors like particle deposition, consolidation of fouling materials on the interior space of the membrane, or formation of a cake layer caused the long period of constant flux.

In relation to total protein content, the least flux decline occurred during filtration of F2, which contained the smallest amount of total protein. This may be attributed to the negligible occurrence of fouling, as the rejection rate is significantly low, below 40% in comparison to other fish species. On the other hand, F5, with the highest protein content, had the typical flux declination pattern, as did F3, F4, and F1. Conversely, F1 demonstrated the highest transmission rate, likely owing to the uniform distribution of protein molecular weight, as evidenced by the SDS-PAGE gel results. F5 exhibited the highest normalised flux, aligning well with the 48%:52% rejection:transmission ratio. Figure 7 depicts a more detailed relationship between flux and rejection values.

The relationship between protein rejection rate and protein flux during ultrafiltration of surimi washwater shows a complex interaction among membrane selectivity and permeability performance across different fish types. In brief, the data demonstrate an inverse relationship between flux and rejection for most fish types, which is considered a significant indication of overall filtration performance. F2 exhibits the lowest flux combined with low rejection, indicating potential membrane fouling or pore blocking that reduces both permeability and selectivity.

F3 presents the most intriguing case with the highest flux paired with moderate rejection. This combination suggests an optimal balance where the membrane maintains satisfactory permeability while still providing reasonable protein retention. The high flux indicates minimal fouling effects, possibly due to the specific protein characteristics of F3 washwater that are less prone to membrane surface interactions or pore clogging. In relation to the SDS-PAGE result, the F3 vibrant

TABLE 3. Protein content collected from the feed (C_F), the permeate (C_p), and the retentate (C_R) from different species of fish tested

Types of fish	C_F (mg/mL)	C_p (mg/mL)	C_R (mg/mL)
F1	1611.25	1273.75	1170.00
F2	1420.00	860.00	1148.75
F3	2312.50	847.50	1902.50
F4	1497.50	1105.00	1297.50
F5	2346.25	1222.50	2228.75

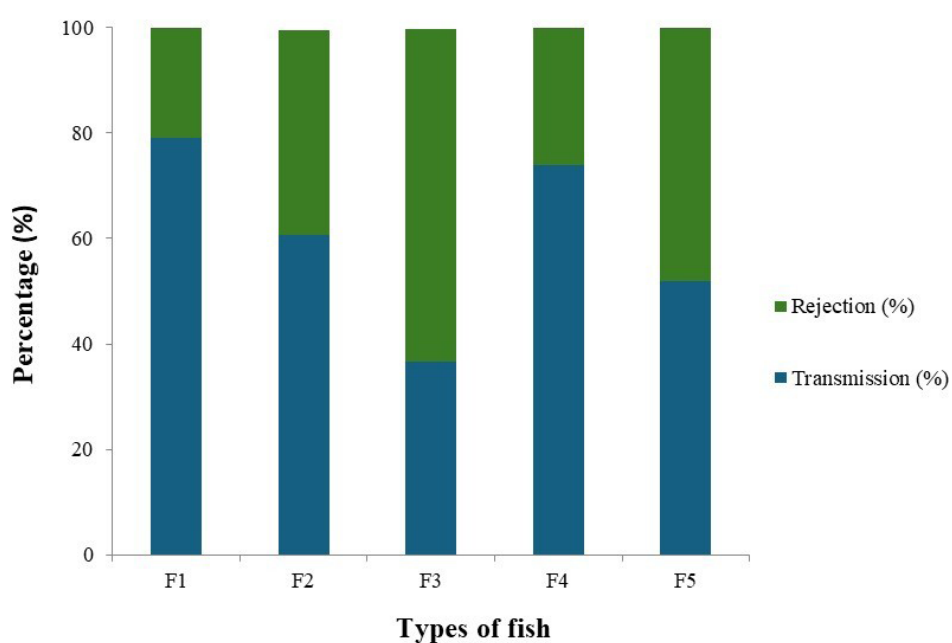


FIGURE 5. Graph of types of fish versus percentage of transmission and rejection

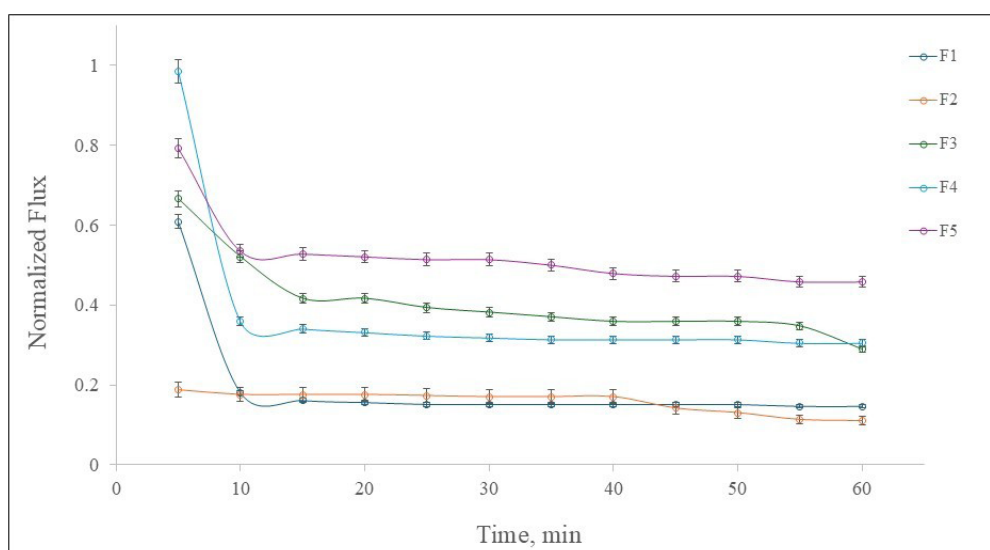


FIGURE 6. Graph of permeate flux for different fish versus time

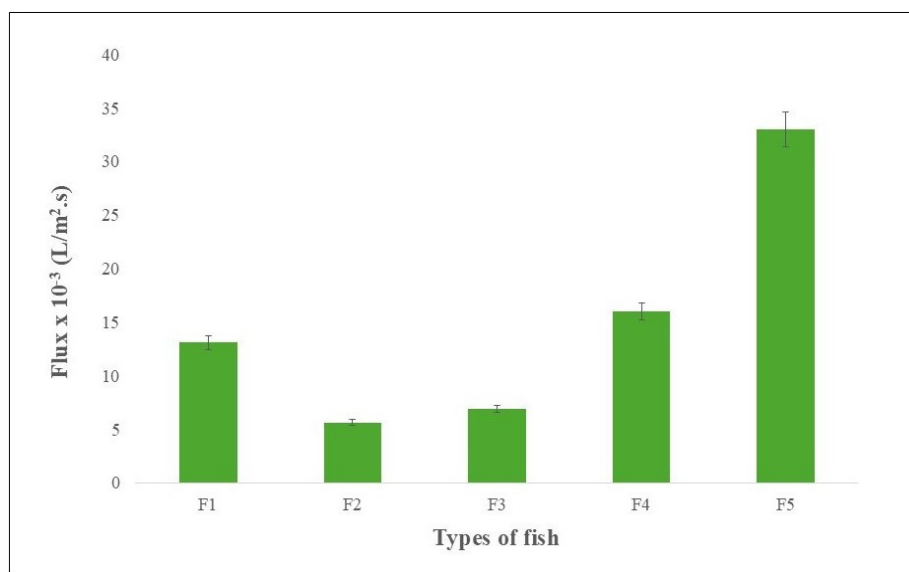


FIGURE 7. Graph of accumulated permeate flux for sample F1 to F5

band of smaller proteins, 66 kDa and below, is a good reason for less fouling effect, as the membrane MWCO is 100 kDa, which is significantly bigger than the size of most proteins contained in F3.

CONCLUSIONS

The results of this study demonstrate that surimi washwater, which contains a substantial number of soluble proteins, represents a promising source of recoverable protein through ultrafiltration. Approximately 80% of the protein was transmitted through the membrane, indicating effective protein permeation with only a small proportion retained in the retentate. This observation suggests that the molecular size of most proteins was smaller than the membrane pore size, allowing efficient passage through the membrane.

Among the five local white-fleshed fish species (F1-F5) evaluated, F5 exhibited the highest total protein concentration in the feed (2346.250 mg/mL), followed by F3, F1, F4, and F2. However, the permeate obtained from F1 contained the highest protein concentration (1273.750 mg/mL), while the retentate from F5 showed the highest protein concentration (2228.75 mg/mL). Based on the transmission and rejection analyses, F1 demonstrated the best membrane separation performance, with the highest protein transmission (79%) and the lowest rejection (21%) among all samples. In terms of membrane performance, F5 achieved the highest permeate flux (0.033 L/m².s), followed by F4, F1, F3, and F2.

SDS-PAGE analysis showed that both the permeate and retentate fractions contained distinct protein profiles,

reflecting the selective separation of proteins according to their molecular sizes. In addition, protease activity varied among the feed, permeate, and retentate streams under both acidic and alkaline conditions, indicating that protein recovery and enzymatic characteristics were influenced by protein solubility associated with acid- and alkali-assisted extraction methods.

Although the findings highlight the potential of ultrafiltration for protein recovery from surimi washwater, several limitations should be addressed in future studies. The use of membranes with smaller molecular weight cut-off (MWCO) values is recommended to improve the retention and separation of low-molecular-weight proteins. Comparative investigations involving pelagic fish and additional white-fleshed fish species would provide a broader understanding of variations in protein recovery efficiency. Furthermore, proteolytic activity should be quantified using a synthetic substrate, such as N α -benzoyl-L-arginine-4-nitroanilide (Bz-L-Arg-4-NA), to enable more accurate and standardized enzyme activity measurements. Finally, comprehensive SDS-PAGE analyses under different experimental conditions should be conducted for all fish species to further characterize protein profiles and evaluate the influence of processing parameters on protein separation.

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REFERENCES

- Ahmed, I., Jan, K., Fatma, S. & Dawood, M.A.O. 2022. Muscle proximate composition of various food fish species and their nutritional significance: A review. *Journal of Animal Physiology and Animal Nutrition* 106(3): 690-719. <https://doi.org/10.1111/jpn.13711>
- Ali, N., Kamarudin, D. & Ismail, N.M. 2017. Minimizing flux decline in surimi wash water ultrafiltration for protein recovery. *Desalination and Water Treatment* 72: 222-227. <https://doi.org/10.5004/dwt.2017.20237>
- Baraiya, K.G., Bojayanai, M., Taral, P.V., Surasani, V.K.R., Krishnamoorthy, E. & Shetty, V. 2023. Utilizing bullseye fish processing frame waste to produce edible proteins and quality assessment of the recovered proteins. *Environmental Science and Pollution Research* 31(53): 62296-62304. <https://doi.org/10.1007/s11356-023-29738-5>
- Batista, I., Pires, C. & Nelhas, R. 2007. Extraction of sardine proteins by acidic and alkaline solubilisation. *Food Science and Technology International* 13(3): 189-194. <https://doi.org/10.1177/1082013207079619>
- Bradford, M.M. 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72(1-2): 248-254. [https://doi.org/10.1016/0003-2697\(76\)90527-3](https://doi.org/10.1016/0003-2697(76)90527-3)
- Chaijan, M., Benjakul, S., Visessanguan, W. & Faustman, C. 2006. Physicochemical properties, gel-forming ability and myoglobin content of sardine (*Sardinella gibbosa*) and mackerel (*Rastrelliger kanagurta*) surimi produced by conventional method and alkaline solubilisation process. *European Food Research and Technology* 222(1-2): 58-63. <https://doi.org/10.1007/s00217-005-0091-1>
- Das, A., Verma, M. & Mishra, V. 2023. Food waste to resource recovery: A way of green advocacy. *Environmental Science and Pollution Research* 31(12): 17874-17886. <https://doi.org/10.1007/s11356-023-27193-w>
- de Oliveira, D.L., Grassi, T.L.M., Paiva, N.M., Santana, B.N., Nakamura, A.A., Bermejo-Poza, R. & Ponsano, E.H.G. 2021. Ultrafiltration for the recovery of proteins from surimi washing water. *Food Science and Technology* 41(suppl 2): 458-464. <https://doi.org/10.1590/fst.30120>
- Ghalamara, S., Brazinha, C., Silva, S. & Pintado, M. 2024. Exploring fish processing by-products as an alternative source of bioactive peptides: A review on extraction and food applications. *Current Food Science and Technology Reports* 2(4): 377-391. <https://doi.org/10.1007/s43555-024-00041-9>
- Hou, Q., Yang, Z., Chen, S. & Pei, H. 2020. Using an anaerobic digestion tank as the anodic chamber of an algae-assisted microbial fuel cell to improve energy production from food waste. *Water Research* 170: 115305. <https://doi.org/10.1016/j.watres.2019.115305>
- Iftikhar, A., Hayat, M.T., Bibi Saima Zeb, B.S.Z., Siddique, M., Ahmed Bhatti, Z., Abbasi, U. & Mahmood, Q. 2023. Anaerobic biological treatment of wastewater from paper recycling industry by UASB reactor. *Sains Malaysiana* 52(4): 1087-1099. <https://doi.org/10.17576/jsm-2023-5204-05>
- Kamarudin, D., Hashim, N.A., Ong, B.H., Faried, M., Suga, K., Umakoshi, H. & Wan Mahari, W.A. 2022. Alternative fouling analysis of PVDF UF membrane for surface water treatment: The credibility of silver nanoparticles. *Journal of Membrane Science* 661: 120865. <https://doi.org/10.1016/j.memsci.2022.120865>
- Kamarudin, D., Ali, N., Ismail, M. & Othman, N. 2015. Fouling evaluation for ultrafiltration of protein-based washwater: A resistance-in-series model approach. *Jurnal Teknologi* 74(7): 41-47. <https://doi.org/10.11113/jt.v74.4695>
- Khatprathum, A., Siriwongpaisaan, P. & Youravong, W. 2010. Concentration of protein in fish mince wash water discharged from surimi processing plant by ultrafiltration. *Desalination and Water Treatment* 21(1-3): 1-7. <https://doi.org/10.5004/dwt.2010.1148>
- Kristinsson, H.G. & Hultin, H.O. 2004. Changes in trout hemoglobin conformations and solubility after exposure to acid and alkali pH. *Journal of Agricultural and Food Chemistry* 52(11): 3633-3643. <https://doi.org/10.1021/jf034563g>
- Lee, H.J., Lee, G-W., Yoon, I.S., Park, S.H., Park, S.Y., Kim, J-S. & Heu, M.S. 2016. Preparation and characterization of protein isolate from Yellowfin tuna *Thunnus albacares* roe by isoelectric solubilization/precipitation process. *Fisheries and Aquatic Sciences* 19: 14. <https://doi.org/10.1186/s41240-016-0014-z>
- Lin, T.M., Park, J.W. & Morrissey, M.T. 1995. Recovered protein and reconditioned water from surimi processing waste. *Journal of Food Science* 60(1): 4-9. <https://doi.org/10.1111/j.1365-2621.1995.tb05594.x>
- Luo, X., Xiao, S., Ruan, Q., Gao, Q., An, Y., Hu, Y. & Xiong, S. 2022. Differences in flavor characteristics of frozen surimi products reheated by microwave, water boiling, steaming, and frying. *Food Chemistry* 372: 131260. <https://doi.org/10.1016/j.foodchem.2021.131260>
- Marshall, A.D., Munro, P.A. & Trägårdh, G. 1993. The effect of protein fouling in microfiltration and ultrafiltration on permeate flux, protein retention and selectivity: A literature review. *Desalination* 91(1): 65-108. [https://doi.org/10.1016/0011-9164\(93\)80047-Q](https://doi.org/10.1016/0011-9164(93)80047-Q)

- Muhamad Nor Iqmal bin Mamat, Hafeedza Abdul Rahman, Noorul Syuhada Mohd Razali, Sharifah Salmah Syed Hussain, Khairul Farihan Kasim & Noor-Soffalina Sofian-Seng. 2025. A review on fish oil extraction from fish by-product as sustainable practices and resource utilization in the fish processing industry. *Sains Malaysiana* 54(1): 165-174. <https://doi.org/10.17576/jsm-2025-5401-13>
- Pham, T.P.T., Kaushik, R., Parshetti, G.K., Mahmood, R. & Balasubramanian, R. 2015. Food waste-to-energy conversion technologies: Current status and future directions. *Waste Management* 38: 399-408. <https://doi.org/10.1016/j.wasman.2014.12.004>
- Rawdkuen, S., Sai-Ut, S., Khamorn, S., Chaijan, M. & Benjakul, S. 2009. Biochemical and gelling properties of tilapia surimi and protein recovered using an acid-alkaline process *Food Chemistry* 112(1): 112-119. <https://doi.org/10.1016/j.foodchem.2008.05.047>
- Rustad, T., Storror, I. & Slizyte, R. 2011. Possibilities for the utilisation of marine by-products. *International Journal of Food Science & Technology* 46(10): 2001-2014. <https://doi.org/10.1111/j.1365-2621.2011.02736.x>
- Sahana, M.D., Balange, A.K., Elavarasan, K., Layana, P., Xavier, K.A.M., Nayak, B.B. & Naidu, B.C. 2025. Molecular and functional analysis of proteases isolated from tropical fish visceral waste via a cost-effective dual-buffer technique. *Waste and Biomass Valorization* 16(6): 3119-3129. <https://doi.org/10.1007/s12649-024-02852-2>
- Shamasundar, B.A. 2022. Surimi and surimi-based products. In *Advances in Fish Processing Technologies*, 1st ed., edited by Majumder, R.K. & Balange, A.K. New York: Apple Academic Press. pp. 31-52. <https://doi.org/10.1201/9781003300595-3>
- Sihono, Purnomo, A.H., Wibowo, S. & Dewi, F.R. 2021. Current (2021) status of surimi industry in Indonesia and possible solutions: A review. *IOP Conference Series: Earth and Environmental Science* 919(1): 012036. <https://doi.org/10.1088/1755-1315/919/1/012036>
- Siti Kudnie Sahari, Amir Maina Butit, Zainab Ngaini, Yanuar Zulardiansyah Arief, Kuryati Kipli, Martin Anyi, Asmahani Awang, Muhammad Kashif, Mohamad Rusop Mahmood, Zaidi Embong, Lilik Hasanah, Abdul Rahman Kram & Marini Sawawi. 2023. Bioelectricity generation from bamboo leaves waste in a double chambered microbial fuel cell. *Sains Malaysiana* 52(6): 1855-1864. <https://doi.org/10.17576/jsm-2023-5206-20>
- Song, Z., Zhang, S., Qi, X., Yin, M. & Wang, X. 2024. Application of ultrasound technology in the washing process of surimi: Improvement of meat yield and gel quality. *Food Biophysics* 19(3): 745-760. <https://doi.org/10.1007/s11483-024-09843-9>
- Tanudjaja, H.J., Anantharaman, A., Ng, A.Q.Q., Ma, Y., Tanis-Kanbur, M.B., Zydney, A.L. & Chew, J.W. 2022. A review of membrane fouling by proteins in ultrafiltration and microfiltration. *Journal of Water Process Engineering* 50: 103294. <https://doi.org/10.1016/j.jwpe.2022.103294>
- Yingchutrakul, M., Wasinnitwong, N., Benjakul, S., Singh, A., Zheng, Y., Mubango, E., Luo, Y., Tan, Y. & Hong, H. 2022. Asian carp, an alternative material for surimi production: Progress and future. *Foods* 11(9): 1318. <https://doi.org/10.3390/foods11091318>
- Zayas, J.F. 1997. *Functionality of Proteins in Food*. Springer Berlin Heidelberg. <https://doi.org/10.1007/978-3-642-59116-7>