Physicochemical and gel-forming properties of protein isolated from salmon, cod and herring by-products using the pH-shift method

LWT - Food Science and Technology 101 (2019) 678–684

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Physicochemical and gel-forming properties of protein isolated from salmon, cod and herring by-products using the pH-shift method

Mehdi Abdollahi*, Ingrid Undeland

Department of Biology and Biological Engineering–Food and Nutrition Science, Chalmers University of Technology, SE 412 96, Gothenburg, Sweden

ARTICLE INFO

Keywords:
Marine by-products
Fish protein isolate
pH-shift method
Solubilization/isoelectric precipitation

ABSTRACT

The impacts of variation in fish filleting by-products origin including white muscle (cod), dark muscle (herring) and farmed fish (salmon) on physicochemical and gel-forming properties of protein recovered using the pH-shift method were studied. The effects of different solubilization pHs (acid or alkaline) on protein yield/composition, and its properties were also studied. Alkaline version (pH 11.5–12.5) resulted in maximum protein yield for the three resources which ranked them as: salmon > herring > cod. Increasing solubilization pH from 11.5 to 12.5 increased protein yield in salmon and herring, while maximum protein yield of cod was obtained at pH 12. However, increasing solubilization pH from 11.5 to 12.5 required a two-fold higher amount of alkali compared to the adjustment to 11.5. All recovered proteins had gel-forming capacity; however, cod gels showed higher WHC and breaking force compared with salmon and herring protein gels. Increasing solubilization pH from 11.5 to 12.5 negatively affected the breaking force and color of the salmon and cod protein gels, but improved heme pigment removal, breaking force and whiteness of herring protein gels. The pH-shift process thus showed good potential for recovering high quality protein from the by-products, but protein solubilization pH should be carefully selected based on the target species.

1. Introduction

The fish processing industry produces large amounts of side streams which normally form more than 50% of the original fish weight. Globally, these by-products are already mainly used in animal feed production or are even wasted (Aspevik, Totland, Lea, & Oterhals, 2016). Increasing demands for seafood products due to increasing world population and awareness about the health effects of seafood in parallel with wild fish supply decline have caused great interest in using fish processing by-products in food production. Some of these by-products, such as head, backbone, tail and trimming, are a good source of high value food-grade products like fish protein, oil and minerals. However, the complex bony nature of these materials and their high heme protein and lipid content have barricaded their successful use in e.g. protein ingredient production (Abdollahi, Marmon, Chaijan, & Undeland, 2016; Aspevik et al., 2016). These challenges have led to continuous research to find methods that can recover proteins from these complex structure, while retaining their functionality.

An alternative to classic meat-bone separation using a belt and drum or conventional washing method, the so-called acid-alkaline solubilization/isoelectric precipitation or pH-shift method patented by Hultin et al. (2001), has shown high potential for protein isolation from non-conventional complex marine resources. In this process, muscle is solubilized in water at low or high pH, < 3.5 or > 11, respectively, which drives the muscle proteins apart from each other. High and low density undissolved material easily be removed, and proteins can be subsequently de-watered by adjusting the pH to a value where they have minimal solubility (pH ~ 5.5). Previous studies have shown that when pH-shift process is used for fish processing by-products, the protein yield of the process and functionality of the recovered protein will be dependent on the quality of the initial raw material, process version (acid or alkaline) and solubilization/precipitation pHs (Abdollahi & Undeland, 2018; Chen & Jaczynski, 2007a; Chomnawang & Yongsawatdigul, 2013; Panpipat & Chaijan, 2017). However, there is no comprehensive report that side by side evaluates the effect of by-products variations caused by fish origin, including salmon (*Salmo salar*), cod (*Gadus morhua*) and herring (*Clupea harengus*) representing dark and white muscle fish as well as fatty fish and lean fish on the yield and quality of the protein isolated using the pH-shift method.

A handful of studies have also reported that increasing solubilization pH may increase the protein yield of the pH-shift process. Beyond that, some studies (Chen & Jaczynski, 2007; Taskaya et al., 2009b) have shown that the effect of very high solubilization pHs on the functional properties of the final protein isolate may be species specific and would

* Corresponding author.
E-mail address: khozaghi@chalmers.se (M. Abdollahi).

https://doi.org/10.1016/j.lwt.2018.11.087
Received 2 March 2018; Received in revised form 24 November 2018; Accepted 28 November 2018
Available online 29 November 2018

be dependent on the refolding capacity of the proteins after experiencing extreme unfolding during the solubilization/isoelectric precipitation process. As the required amount of alkaline/acid to adjust the pH to values higher or lower than 11.5 or 3, respectively, increase logarithmically, the solubilization pH however becomes important from economic and environmental perspectives (Nolsøe & Undeland, 2009). However, to the best of our knowledge there is no systematic report considering the effect of different solubilization pHs on the consumed amount of alkaline/acid, protein yield or the physico-chemical quality of the recovered protein under the same lab condition.

Thus, the present study was aimed to evaluate how compositional variations caused by by-product origin, including white muscle/lean fish (cod), dark muscle/fatty fish (herring) and farmed salmonid fish (salmon), can affect physicochemical and gel-forming properties of protein recovered using the pH-shift method. In addition, the effect of different solubilization pHs on the consumption of acid/alkali, protein yield and the protein isolate quality of the three resources was studied. The two solubilization pHs providing the maximum protein yield for each species was used to investigate the functional and gel-forming capacity of the recovered protein isolate were evaluated.

2. Materials and methods

2.1. Fish samples preparation

Fresh filleting rest raw materials (head and tail on backbone) of cod and salmon (from 10 fish) were provided by Fisk Idag AB (Gothenburg, Sweden). Fresh herring filleting by-products (head, backbone and tail from more than 100 fish) were also provided by Scandic Pelagic AB. The samples were caught less than 2 days before arrival for grinding and were immediately grinded using a table top meat mincer (C/E22 N, Minerva Omega group, Italy) equipped with a plate with 3 mm holes and pooled completely. All stops of transportation, mincing and packaging were conducted in cold condition and beside ice to keep the temperature below 5 °C. Finally, the mince was frozen at −80 °C in plastic zip-lock bags for further use. Storage at −80 °C did not exceed 2 months.

2.2. Protein isolation using the pH-shift process

Minced by-products from the different species were subjected to pH-shift processing following the main steps reported by Undeland, Kelleher, and Hultin (2002b), but with some modifications. To evaluate protein isolation yield, 100 g fish mince was homogenized with 6 vol of cold distilled water for 1 min at speed 4 using a Polytron Homogenizer (IKA, Germany). Then, the homogenate was adjusted to acidic (2, 2.5, 3) or alkaline (11.5, 12, 12.5) pH using 2 M HCl or NaOH, respectively, with a titrator (907 Titrando, Metrohm AG, Zurich, Switzerland) automatically in set pH mode with a maximum titration rate. The pH-adjusted homogenate was incubated in ice for 10 min and then centrifuged at 8500 g in a Thermo Scientific Sorvall LYNX Superspeed Centrifuge (Thermo Fisher Scientific, Waltham, USA) for 20 min at 4 °C. The mid layer containing soluble proteins was separated from floating fat layer and insoluble residues. The pH of separated protein layer was then adjusted to pH 5.5 with 10 min incubation time at pH 5.5 on ice followed with a second centrifugation step at 8500 × *g* (4 °C, 20 min) to dewater the precipitated proteins.

To evaluate functional properties of the protein isolates, the same process was conducted by mixing 700 g of minced by-product with 4.2 L water. The pH of the slurry after homogenization was adjusted to the two pHs, resulting in maximum protein yield for each species, and the pH-shift process was continued as described above. Cold NaOH (2N) (~4 °C) was used to adjust the pH of recovered protein isolates to 7 under cold condition (< 4 °C) followed by addition of 4% (w/w) sucrose and 4% (w/w) sorbitol and frozen storage at −80 °C until used. The storage time did not exceed 1 month.

2.3. Measurement of protein solubility and yield

Protein solubility and yield in both the acid process version (pH 1.5, 2 and 2.5) and the alkaline process version (11.5, 12 and 12.5) was studied by measuring protein content using the Lowry method as modified by Markwell, Haas, Bieber, and Tolbert (1978) in the initial homogenate at target pH (H), first supernatant (S_1) and second supernatant (S_2). Calculation was done using equation number 1 and 2.

$$solubility\cdot(\%) = \frac{Protein\ \ content\ of\ S1\left(\frac{mg}{ml}\right)}{Protein\ \ content\ of\ H\left(\frac{mg}{ml}\right)} \times 100 \quad (1)$$

The protein yield of the entire pH-shift process was calculated using equation number 2.

$$Yield(\%) = \frac{Protein\ content\ of\ S1\,(mg) - Protein\ content\ of\ S2\,(mg)}{Protein\ \ content\ ofH\ (mg)} \times 100 \quad (2)$$

2.4. Characterization of recovered protein isolate

2.4.1. Composition analysis

Protein content of the RRM and their corresponding isolate samples from the alkaline process version was measured using the Lowry method as modified by Markwell et al. (1978). Total fat content was measured according the method of Lee, Trevino, and Chaiyawat (1995) as modified by Undeland, Hultin, and Richards (2002a). For initial minced raw materials, a chloroform:methanol ratio of 2:1 (v/v) was used, while for the protein isolates a 1:1 ratio was used. Moisture and ash contents were measured by calculating weight difference after drying the samples at 105 °C and 500 °C, respectively, for 24 h.

2.4.2. Total heme pigment measurement

Total heme pigment of protein isolates recovered from different fish by-products was measured using the Hornsey method (Hornsey, 1956) with slight modification as previously explained by Abdollahi et al. (2016).

2.5. Gel preparation from recovered protein isolates

Blend protein isolate was partially thawed under running tap water until core temperature reached 0 °C followed by cutting into small pieces and grounding for 2 min in a chopper (Moulinex Moulinette presse-purée, Moulinex S.A., Paris, France). The moisture content of the samples was adjusted to 80% by addition of melting ice and 2 min chopping that followed by 5 more min chopping in the presence of 2% w/w of NaCl to develop the homogeneous paste. It was followed by stuffing the paste into stainless steel tubes with a diameter of 15 mm. Both ends of the tubes were closed and sealed tightly and were subjected to one-step heating at 90 °C for 20 min in a water bath. The gels were finally removed from the bath and immediately cooled in iced water to stop further heating, and stored overnight at 4 °C prior to analysis.

2.6. Characterization of gels made of fish protein isolate

2.6.1. Measurement of water holding capacity

A 2 g (X) properly chopped gel sample was used to measure water holding capacity (WHC) based on their gravimetrical method of Cardoso, Mendes, Vaz-Pires, and Nunes (2009). Gel sample was wrapped within two layers of pre weight (Y) Filter Paper (Sigma-Aldrich, Germany), placed in a 50 ml centrifuge tube and centrifuged at 3000 × *g* for 10 min at 20 °C. Then, the filter papers were weighed again (Z) after removing the sample. WHC (%) was calculated by equation (1) and presented as percentage.

$$WHC = \frac{X \times \left(\frac{M}{100}\right) - (Z - Y)}{X - \left(\frac{M}{100}\right)} \times 100 \quad (3)$$

where M is initial moisture (%).

2.6.2. Texture analysis of gel

Puncture and texture profile analyses (TPA) tests were used to measure textural properties of the gels as explained by (Abdollahi, Rezaei, Jafarpour, & Undeland, 2017). After equilibration at room temperature (23–25 °C) for 2 h, 10 cylinder-shaped gel samples with a length of 1.5 cm were prepared. Then, the puncture test was conducted with a 5-mm spherical probe using a 5-kg load cell, at depression speed of 60 mm/min using a TA.HDi texture analyzer (Stable Micro Systems, UK). TPA was conducted by twice compression (40%) of gel samples with a 25-mm cylindrical probe with 5 s rest between the two compression cycles, at depression speed of 60 mm/min. Both tests were conducted with 5 replicates.

2.6.3. Color measurement

Color attributes of small pieces (1.5 cm in diameter and 1.5 cm in thickness) of gel samples were measured in the CIE L*a*b* color space as explained by Abdollahi, Rezaei, Jafarpour, & Undeland (2018). A Minolta colorimeter (CR-400, Konica Minolta Sensing, Japan) probe was directly held against the bottom of a flat polystyrene plate containing the samples. The color parameters were recorded with 5 replicate for each gel at different locations of the plates and were used for calculation of whiteness using equation (2):

$$Whiteness = 100 - \sqrt{(100 - L)^2 + a^2 + b^2} \quad (4)$$

2.6.4. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The polypeptide pattern of by-products, proteins and gel samples was investigated using SDS-PAGE according to the method of Laemmli (1970). Initially, 27 ml of 5% SDS solution was added to 3 g of each gel and homogenized in an Ultra-Turrax homogenizer at speed 3 for 2 min and then heated at 85 °C for 1 h. After cooling, the dissolved samples were centrifuged at 5000 × *g* for 20 min to remove undissolved residuals. The supernatant was mixed with an equal amount of Laemmli buffer (Bio-Rad, USA) containing 5% βME and then boiled for 5 min. Ten µl of protein ladder (prestained dual color standard, 10–250 kDa, Bio-Rad, USA) and 15 µg of protein from each sample were loaded onto 4–20% precast mini linear gels (Bio-Rad, USA). Electrophoresis was conducted at a constant voltage of 205 V, using a Mini Protein II unit (Bio-Rad, USA). After separation, 0.02% (w/v) Coomassie Brilliant Blue R-250 in 50% (v/v) methanol and 7.5% (v/v) acetic acid was used for staining, and destaining was conducted with 50% methanol (v/v) and 7.5% (v/v) acetic acid for 1 h. Finally, the gel was scanned in a GS-800 Calibrated Densitometer (Bio-Rad, USA).

2.7. Statistical analysis

Significant differences between the variables was analyzed using One-way analysis of variance (ANOVA) and Duncan's multiple range test. Differences with a $p < 0.05$ were considered significant and all data were reported in the form of mean ± standard deviation. All experiments were run at least in triplicate (n = 3).

3. Results and discussion

3.1. Protein solubility and yield during the pH-shift processing

In general, with the three studied resources, the alkaline process version resulted in higher protein yield compared with the acid process version (Fig. 1). As can be seen, protein solubility was highest for herring by-products (90.10%), while maximum total protein yield was obtained for salmon by-products (75.49%), both at solubilization pH of 12.5. This means that despite the high solubility of the herring proteins at alkaline pH, they were not fully recoverable during the next steps of the pH-shift processing. This can be also related to the higher amount of sarcoplasmic proteins, such as pigments and enzymes in the herring samples, which can be partially recovered during the pH-shift process and may stay solubilized at the second supernatant (Marmon & Undeland, 2010). Maximum protein solubility and yield of cod by-products were observed at pH 12, 65.90% and 60.09%, respectively, which were lower than maximum solubility and yield obtained for salmon and herring RRM, and occurred at a lower pH. The lower yield could be due to the large volume of sediment obtained in the first

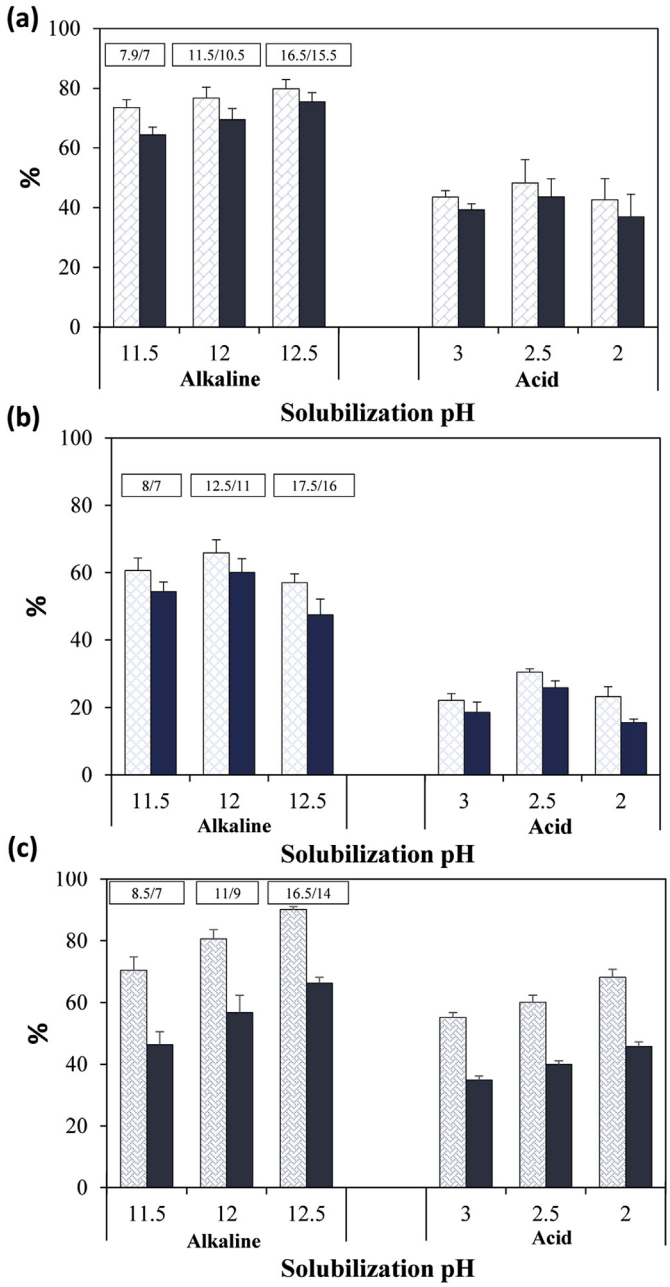

Fig. 1. Protein solubility and yield of pH-shift processing of salmon (a), cod (b) and herring (c) by-products at acidic and alkaline pHs. Numbers in the rectangles show the amount of 2 N alkaline/acid (ml/100 g muscle) used for pH adjustments. Light and dark bars represent protein solubility and yield, respectively.

centrifugation, trapping a lot of the solubilized proteins. In addition, protein isolation yield increased with increasing the solubilization pH between 11.5 and 12.5, and the maximum yield for salmon, cod and herring was observed at 12.5, 12 and 12.5, respectively. Normally, increasing the pH of the homogenate increases the electrostatic charges of the proteins and improves their interaction capacity with the water (solubility), which subsequently increases protein yield of the process. However, composition of the proteins available in the used raw material, their amino acid composition and their conformational situation will be also important for the solubilization capacity of the proteins at a specific pH. This may explain the differences observed in the solubility of the different resources used in the present study. A wide range of total protein yields has been reported for the pH-shift processing of by-products of different fish species including trout (90%) (Chen & Jaczynski, 2007a), silver carp (70.92%) (Zhong et al., 2016), rohu (32%) (Surasani, Tyagi, & Kudre, 2016), whitemouth croaker (64.1%) and Argentine anchovy (63.7%) (Freitas, Cortez-Vega, & Prentice, 2016).

The required amount of alkaline and acid for solubilization and precipitation of protein or vice versa per 100 g of the different studied resources is also shown in Fig. 1. As can be seen, for example, the required amount of alkali (2 N) to adjust the pH of 100 g RRM from its native pH (~7) to pH 11.5 was 7.9–8.5 ml, but the required amount to adjust the pH to 12.5 was 16.5–17.5 ml. This means that a two fold higher amount of alkaline and acid will be required to increase the solubilization pH to 12.5 compared to 11.5 to readjust to 5.5. The higher required amount of alkali might be related to the conformational changes of the proteins at pHs higher than 11.5 causing the exposure of the buried groups in the protein structure. This increases the positive net charge of the protein, which will need a higher amount of alkali for neutralization. It is important from economic and environmental perspectives to know if the higher amount of alkaline and acid results in considerably higher yield or not.

3.2. Proximate composition of the by-products and protein isolates

Proximate composition of the protein isolates from three different resources was only measured after alkaline processing as this version gave highest yield. Protein isolates of the three different resources was significantly different form their original raw material (Table 1). Protein was concentrated in the protein isolates compared to the original by-products, especially those of cod and herring. The increase in the concentration was from 50 to 63 g/100 g in RRM to 67–89 g/100 g in the protein isolates. Increasing the solubilization pH also further increased protein content in the protein isolate. However, the moisture content of the isolate was higher than the initial by-products in all three studied resources. The final ash content obtained here (2.4–3.3 g/100 g DW) was significantly lower than that found in protein isolated from by-products of silver carp (3.5–4.3 g/100 g) (Zhong et al., 2016), but higher than protein isolates from trout (1.3–2.1 g/100 g) (Chen & Jaczynski, 2007a). The by-products contained high amount of ash (12–28 g/100 g), which reflects their bony and complex structure, especially that of cod by-products. The ash content was drastically reduced in the protein isolates to less than 3.4 g/100 g. This shows high capacity of the pH-shift processing in removing impurities during the first centrifugation from the By-products, despite their complex nature.

Lipid content was lowered by 32–36% in the protein isolate of salmon and 57–63% in the protein isolate of herring compared with their by-products. Similarly, Marmon and Undeland (2010) reported fat removal of 50% for gutted herring during the pH-shift processing. The difference observed in the fat reduction of the two species can be related to the stability of the fat layer formed after the first centrifugation. Centrifugation of herring homogenate resulted in a stable and easily separable thick fat layer, while that of salmon resulted a loose and thin fat layer with high risk of mixing with the protein fraction during separation by filtration. Differently from the fatty species, lipid content in the cod protein isolate increased by 87–119% compared with its by-products. The pH-shift processing of cod resulted no fat layer and retained in the protein fraction. Thus, it seems that since a high amount of impurities was removed as sediment during the first centrifugation, the fat accumulated on a relative level in the final protein isolate, which resulted in higher fat content in the final protein isolate compared with the cod by-products. Similarly, Pires et al. (2012) found higher amount of fat in the protein powders developed from Cape hake compared with its sawdust by-product. Increasing solubilization pH increased fat removal with all three studied resources. This might be related to increasing solution polarity by increasing the pH and likely reducing the homogenate viscosity, which facilitates the separation of the fats and formation of floating layer, named reducing emulsion stability.

3.3. Total heme pigment content of the protein isolates

Total heme pigment content of salmon protein isolates was significantly lower than its rest raw material. However, total heme pigment content of cod and herring protein isolates was slightly higher than their initial raw material. Herring protein isolates contained a threefold to fivefold higher amount of heme pigment (58–98 μmol/100 g) compared to the two other protein isolates (Fig. 2). In general, success in removing heme pigments during the classic surimi making process (washing process) will be dependent on the type of species and muscle, storage time and process condition (Chaijan, Benjakul, Visessanguan, & Faustman, 2006). Since the used biomasses in this study were a mixture of by-products contaminated with a high amount of blood pigments, the low amount of heme pigments in the cod and salmon may reflect good efficacy of the pH-shift process in removing the pigments. Abdollahi et al. (2016) showed that the alkaline process version of the pH-shift method could remove up to 77% of heme pigments compared to the used raw material, by keeping the heme protein solubilized in the second supernatant. However, the authors used cod mince model without fat or bone residue. Concentration of heme

Table 1
Proximate composition (g/100 g) of salmon, cod and herring by-products and their protein isolates.

	Moisture	Fat	Protein	Ash
Salmon by-products	61.80 ± 2.40^{f}	41.25 ± 2.08^{a}	50.70 ± 3.80^{c}	12.36 ± 1.19^{c}
Cod by-products	78.14 ± 0.50^{d}	4.63 ± 1.25^{g}	63.67 ± 1.71^{b}	28.87 ± 2.11^{a}
Herring by-products	72.23 ± 0.13^{e}	31.98 ± 1.66^{b}	63.31 ± 2.27^{b}	14.53 ± 0.16^{b}
Salmon protein-pH 12	84.74 ± 0.97^{c}	32.06 ± 0.97^{b}	67.74 ± 5.82^{b}	2.44 ± 0.04^{e}
Salmon protein-pH 12.5	83.64 ± 0.08^{c}	26.33 ± 0.53^{c}	69.71 ± 7.78^{b}	2.79 ± 0.00^{e}
Cod protein-pH 11.5	89.54 ± 0.16^{a}	10.14 ± 0.28^{ef}	84.55 ± 2.92^{a}	3.30 ± 0.19^{d}
Cod protein-pH 12	87.40 ± 0.01^{b}	8.66 ± 0.04^{f}	86.87 ± 3.80^{a}	3.09 ± 0.07^{d}
Herring protein-pH 12	84.82 ± 0.21^{c}	13.44 ± 0.03^{d}	86.09 ± 3.16^{a}	3.15 ± 0.30^{d}
Herring protein-pH 12.5	83.50 ± 0.65^{c}	11.74 ± 0.51^{de}	89.13 ± 5.39^{a}	3.19 ± 0.02^{d}

Different small letters in each column show significant differences ($p \leq 0.05$). pH 11.5–12.5 shows the used solubilization pHs.
Protein, fat and ash content are presented based on g/100 of dry weight.

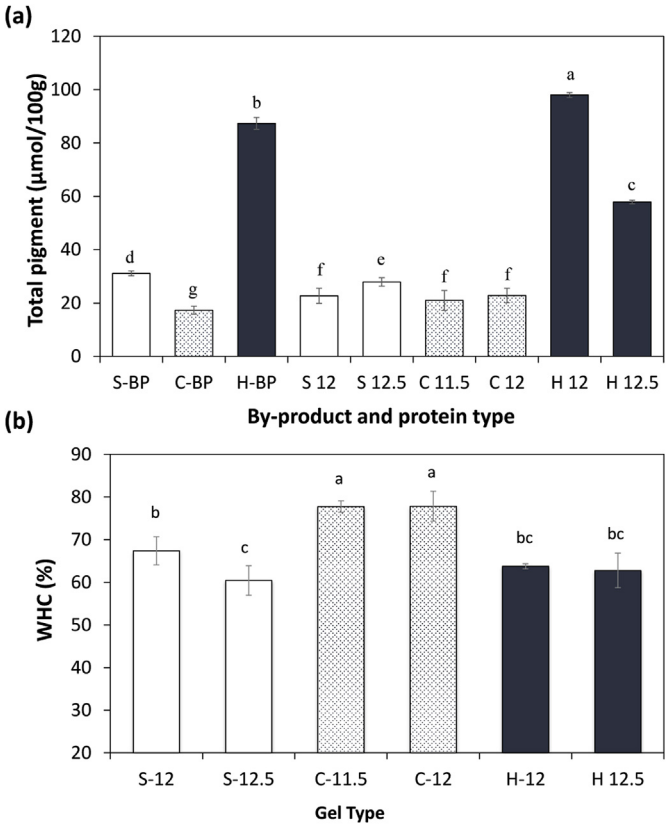

Fig. 2. Total heme pigment (a) and water holding capacity (b) of protein isolates and gels. S—BP, C—BP and H-BP: salmon by-product, cod by-product and herring by-product, respectively; S12: S12 or 12.5: Salmon protein isolate solubilized at pH 12 or 12.5; C 11.5 and 12: Cod protein isolate solubilized at pH 11.5 or 12; H 12 and 12.5: Herring protein isolate solubilized at pH 12 or 12.5.

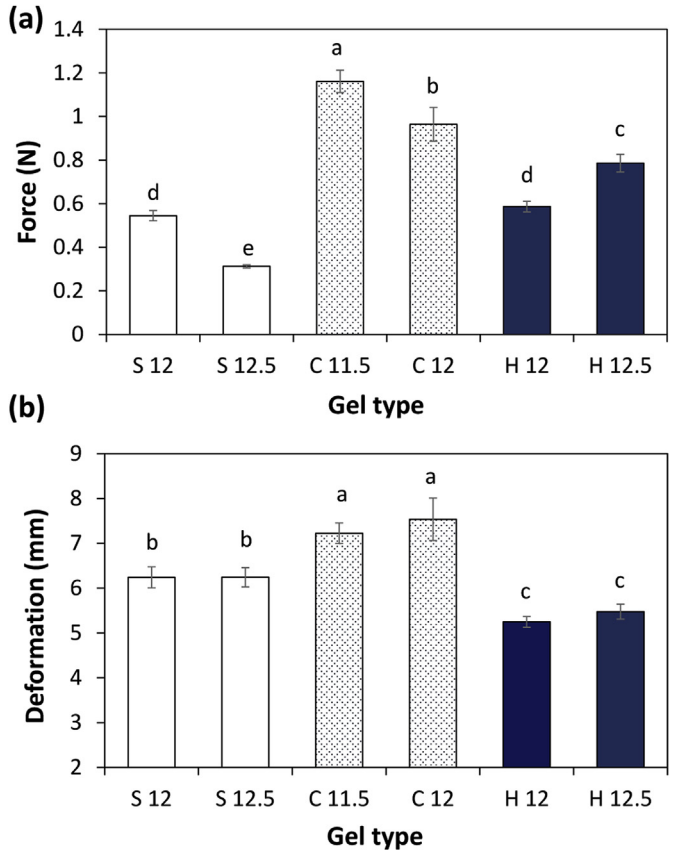

Fig. 3. Breaking force (a) and deformation (b) of gels made of protein isolate from salmon, cod and herring. S12 and 12.5: Salmon protein isolate solubilized at pH 12 or 12.5; C 11.5 and 12: Cod protein isolate solubilized at pH 11.5 or 12; H 12 and 12.5: Herring protein isolate solubilized at pH 12 or 12.5.

pigments during the pH-shift process when using complex rest raw materials might be related to removal of bone and fat with a higher ratio during first centrifugation compared with heme removal, resulting higher final concentration of heme pigments in the final isolate which is less studied. The higher amount of total heme pigments observed in the herring protein isolate can be also related to the higher amount of hemoglobin in the light muscle of this species compared to cod and salmon. Interestingly, application of higher solubilization pH resulted in a considerably lower amount of heme pigments in the final protein isolate of herring RRM, which did not coincide with results obtained from the salmon and cod protein isolates. These results might be related to lesser-known differences in the behavior of heme proteins of these three species during alkaline solubilization and acid precipitation in the presence of myofibrillar proteins.

3.4. Water holding capacity of the gels

In general, WHC of the gels produced from protein isolates recovered from the by-products of the three resources at pH 12 (67–77%) was in the range of that previously reported for gels made of fish protein isolate from gutted kilka (*C. cultiventris*) and silver carp (73–82%) (Abdollahi et al., 2017). As can be seen in Fig. 2b, gels made of cod protein isolate showed significantly higher WHC compared to gels from salmon and herring protein isolates. This might be explained by the fact that macroscopic properties of gels like WHC depend on the balance between protein-protein and protein-water interactions in the gel and their absolute amounts (Romero, Cordobés, Guerrero, & Puppo, 2014). Normally, better organized three-dimensional gel structures result in higher WHC, which is influenced by many factors, such as the type of the formed bands, the amount of sarcoplasmic proteins and fat, conformational changes of proteins and its pH (Chen et al., 2014). Thus, the better WHC capacity of cod protein isolate might be related to its better gel-forming capacity due to a lower amount of fat compared to salmon protein isolate (Table 1) and lower amount of heme pigments (Fig. 2a) compared with the herring protein isolate. Increasing solubilization pH from 12 to 12.5 reduced WHC of gel made of salmon protein isolate, while doing so did not affect gels from the other resources. This might be related to lower refolding capacity of proteins recovered from salmon after experiencing unfolding at extreme pHs, compared to proteins of cod and herring. Partial refolding of the proteins may cause exposure of hydrophobic sites, which could reduce the WHC of the gel network (Abdollahi et al., 2017).

3.5. Textural properties of the gels

Results of breaking force and deformation of gels made of protein isolate of different resources are shown in Fig. 3a and b. All protein isolates recovered at the two solubilization pHs providing highest yields had gel-forming capacity. In contrast, Chen and Jaczynski (2007a) reported that protein isolate recovered from trout by-products failed to gel because of high proteolytic enzyme activity. Panpipat and Chaijan (2017), on the other hand reported acceptable gel-forming capacity for snapper head protein isolate. Gels made of cod protein isolates recovered after solubilization at pH 11.5 and 12 showed significantly higher breaking force and deformation than salmon and herring protein gels. Additionally, there was no significant difference between the breaking force of the gels produced from salmon and herring protein isolates recovered at pH 12. The higher breaking force and deformation observed in the cod gel might be related to the lower fat content and heme pigment content measured in the cod protein isolate compared

with salmon and herring protein isolates, respectively (Chaijan, Panpipat, & Benjakul, 2010). Since lipid remaining in the protein isolate may interfere with actomyosin crosslinking during gel formation, the lower breaking force observed in the salmon gel can be related to its considerably higher amount of fat (Table 1). The substantially higher amount of heme pigments measured in herring protein isolate as shown in Fig. 2a may explain its lower breaking force as well. It is known that sarcoplasmic proteins including heme pigments lack gel-forming capacity and may also interfere with actomyosin gel matrix formation and adversely affect gel breaking force and deformation (Chaijan et al., 2006).

Increasing solubilization pH reduced the breaking force of gels made of salmon and cod protein isolate, while it increased the breaking force of herring gels. The reduction might be attributed to the extreme denaturation of proteins at higher solubilization pHs, resulting in less reversible unfolding after isoelectric precipitation and neutralization (Rawdkuen, Sai-Ut, Khamsorn, Chaijan, & Benjakul, 2009). The increase observed in the breaking force of herring gel by increasing solubilization pH might be related to the considerable reduction of heme pigment content in the herring isolate recovered at pH 12.5.

To better understand the gels' textural properties, TPA of protein isolate-based gels developed from different resources are summarized in Table 1, Supplementary. Results of the hardness of the gels coincide with their breaking force, except for the higher hardness observed for the herring gel produced from protein isolate recovered at pH 12.5. To summarize the presented TPA parameters, gels made of cod protein isolate resulted in the best textural properties followed by salmon and then herring resulted in the worst. Although herring gels showed high hardness, other parameters revealed that gels were less elastic, which might reflect their poor gel structure.

3.6. Color of the gels

Highest whiteness was measured in gels made of salmon protein isolates (67.16), which was significantly higher than the whiteness of cod (56.99) and herring (482) protein gels (Table 2). The whiteness data corresponded to what previously reported for gels made of protein isolate from rohu by-products (41–52), but was higher than snapper head (27–34) (Panpipat & Chaijan, 2017). The lowest whiteness was measured in herring protein gels, which was most likely related to the high amount of heme pigment remaining in the herring protein isolate. These pigments can be oxidized by alkaline used during the pH-shift process at pHs higher than 11.5 to yield brown methemoglobin or metmyoglobin (Abdollahi et al., 2016). Since salmon and cod protein isolate had almost similar amounts of heme pigments, the higher whiteness of the salmon gel compared to cod gel can be related to its higher fat content. Such whiteness enhancement has been attributed to light scattering that results from the emulsion created when oil is comminuted with fish muscle proteins and water (Chen & Jaczynski, 2007a). Increasing solubilization pH reduced the whiteness of salmon and cod gels, which might be related to the reduction in their fat content or further oxidation of heme pigments caused by higher solubilization pH (Abdollahi et al., 2016). However, increasing solubilization pH improved the whiteness of the herring gel, which could be related to the lower amount of heme pigments measured in the herring protein isolate recovered at pH 12.5.

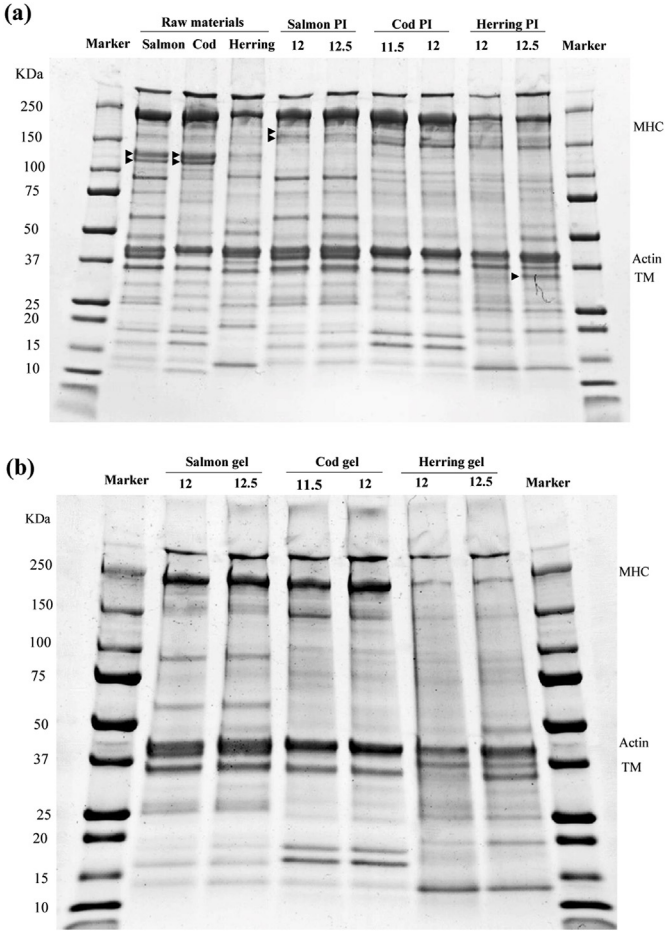

Fig. 4. Polypeptide pattern of salmon, cod and herring by-products and their protein isolates (a) and their gels (b). PI: protein isolate. Numbers from 11.5 to 12.5 on the upper side of the gels show solubilization pH.

3.7. Polypeptide pattern of the protein isolates and gels

The polypeptide pattern of by-products and protein isolate of salmon, cod and herring produced after solubilization at two different pHs are shown in Fig. 4a. With salmon and cod samples, either raw materials or protein isolates, myosin heavy chain (MHC) (~205 kDa) was the most abundant polypeptide. Almost all major bands found in the raw material of the three resources were recovered in the final protein isolate, except two bands between 100 and 150 kDa present in the raw materials, which were not detectable in the final protein isolates (see arrows in Fig. 5a). A dark shadow below the MHC in the protein isolates of the three resources is also seen, which is not detectable in the polypeptide pattern of their raw materials. This might

Table 2
Color properties of gels made of protein isolates from salmon, cod and herring by-products.

	L	*a*	*b*	Whiteness
Salmon gel-pH 12	68.93 ± 0.21 ^{a}	0.16 ± 0.08 ^{c}	10.64 ± 0.23 ^{a}	67.16 ± 0.13 ^{a}
Salmon gel-pH 12.5	65.92 ± 0.35 ^{b}	−0.81 ± 0.08 ^{d}	10.73 ± 0.23 ^{a}	64.26 ± 0.27 ^{b}
Cod gel-pH 11.5	58.07 ± 0.28 ^{c}	−1.58 ± 0.04 ^{e}	9.44 ± 0.15 ^{b}	56.99 ± 0.29 ^{c}
Cod gel-pH 12	56.60 ± 0.46 ^{d}	−1.41 ± 0.09 ^{e}	8.77 ± 0.29 ^{c}	55.70 ± 0.41 ^{d}
Herring gel-pH 12	50.48 ± 0.25 ^{f}	1.12 ± 0.04 ^{a}	8.01 ± 0.23 ^{d}	49.82 ± 0.21 ^{f}
Herring gel-pH 12.5	52.63 ± 0.91 ^{e}	0.77 ± 0.32 ^{b}	8.49 ± 0.61 ^{c}	51.87 ± 1.00 ^{e}

Different small letters in each column show significant differences ($p \leq 0.05$). pH 11.5–12.5 shows the used solubilization pHs.

reflect slight proteolysis caused by proteoleic enzymes (Yongsawatdigul & Park, 2004). The maximum intensity of the MHC band was seen in cod raw material and cod protein isolate recovered at pH 11.5. Since MHC plays the most important role in gel formation and its characteristics, this may explain the higher WHC and breaking force observed in the cod gels. Herring raw materials and protein isolates showed considerably lower MHC band intensity, which may also explain its poor gel properties. This low MHC content might be attributed to the high degree of hydrolysis that has occurring already before the pH-shift process i.e. during pre-processing storage in dark muscle species like herring. Thus, the lower quality of the herring protein isolate might not only be just related to what happens during the pH-shift process, it might also be related to the nature of this species. The only difference in polypeptide patterns seen after solubilization at 2 different pHs was for herring where more actin and tropomyosin retained after solubilization at pH 12.5. At pH 12, there seemed to be some degradation of these polypeptides. The polypeptide pattern of the thermal-induced gels showed lower intensity of MHC compared with the corresponding initial raw materials and protein isolates (Fig. 4b). This may suggest polymerization of the polypeptides (Panpipat & Chaijan, 2017) during gelation.

4. Conclusions

Maximum protein yield for the three resources was obtained using the alkaline process version which ranked the three resources as: salmon > herring > cod. Increasing solubilization pH from 11.5 to 12.5 increased protein yield in salmon and herring, while maximum protein yield of cod was obtained at pH 12. However, increasing solubilization pH from 11.5 to 12.5 required a two-fold higher amount of alkaline compared to the adjustment to 11.5. Although salmon and herring protein isolates showed lower fat content compared to their original raw material, the opposite was true for cod protein isolate. All recovered proteins had gel-forming capacity; however, cod gels showed higher WHC and breaking force compared with salmon and herring protein gels. Increasing solubilization pH negatively affected the breaking force and color of the gels made of salmon and cod protein isolates, but improved heme pigment removal, breaking force and whiteness of herring gels. In conclusion, the pH-shift process showed good potential for recovering high quality protein from fish filleting by-products of the studied species (especially cod and salmon), but solubilization pH should be carefully selected based on the species used.

Acknowledgements

The authors would like to thank VINNOVA for financial support of the research (project# 2014-03469). The authors are also grateful to Fisk Idag AB and the Sweden Pelagic AB for kindly providing the fish processing by-products.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.lwt.2018.11.087.

References

Abdollahi, M., Marmon, S., Chaijan, M., & Undeland, I. (2016). Tuning the pH-shift protein-isolation method for maximum hemoglobin-removal from blood rich fish muscle. *Food Chemistry, 212*, 213–224.

Abdollahi, M., Rezaei, M., Jafarpour, A., & Undeland, I. (2017). Dynamic rheological, microstructural and physicochemical properties of blend fish protein recovered from kilka (Clupeonella cultriventris) and silver carp (Hypophthalmichthys molitrix) by the pH-shift process or washing-based technology. *Food Chemistry, 229*, 695–709.

Abdollahi, M., & Undeland, I. (2018). Structural, functional and sensorial properties of protein isolate produced from salmon, cod and herring by-product. *Food and Bioprocess Technology, 11*(9) 1733-1724.

Aspevik, T., Totland, C., Lea, P., & Oterhals, Å. (2016). Sensory and surface-active properties of protein hydrolysates based on Atlantic salmon (Salmo salar) by-products. *Process Biochemistry, 51*(8), 1006–1014.

Cardoso, C., Mendes, R., Vaz-Pires, P., & Nunes, M. L. (2009). Effect of dietary fibre and MTGase on the quality of mackerel surimi gels. *Journal of the Science of Food and Agriculture, 89*(10), 1648–1658.

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.

Chaijan, M., Panpipat, W., & Benjakul, S. (2010). Physicochemical and gelling properties of short-bodied mackerel (Rastrelliger brachysoma) protein isolate prepared using alkaline-aided process. *Food and Bioproducts Processing, 88*(2–3), 174–180.

Chen, X., Chen, C. gui, Zhou, Y. zi, Li, P., jun, Ma F., Nishiumi, T., et al. (2014). Effects of high pressure processing on the thermal gelling properties of chicken breast myosin containing κ-carrageenan. *Food Hydrocolloids, 40*, 262–272.

Chen, Y.-C., & Jaczynski, J. (2007). Protein recovery from rainbow trout (Oncorhynchus mykiss) processing byproducts via isoelectric solubilization/precipitation and its gelation properties as affected by functional additives. *Journal of Agricultural and Food Chemistry, 55*(22), 9079–9088.

Chen, Y. C., & Jaczynski, J. (2007a). Gelation of protein recovered from whole antarctic krill (Euphausia superba) by isoelectric solubilization/precipitation as affected by functional additives. *Journal of Agricultural and Food Chemistry, 55*(5), 1814–1822.

Chomnawang, C., & Yongsawatdigul, J. (2013). Protein recovery of Tilapia frame by-products by pH-shift method. *Journal of Aquatic Food Product Technology, 22*(2), 112–120.

Hornsey, H. C. (1956). The colour of cooked cured pork. I.—estimation of the Nitric oxide-Haem Pigments. *Journal of the Science of Food and Agriculture, 7*(8), 534–540.

Hultin, H. O., Kelleher, S. D., Feng, Y., Richards, M. P., Kristinsson, H., Undeland, I., et al (2001). High efficiency protein extraction. Google Patents.

Lee, C. M., Trevino, B., & Chaiyawat, M. (1995). A simple and rapid solvent extraction method for determining total lipids in fish tissue. *Journal of AOAC International, 79*(2), 487–492.

Markwell, M. A., Haas, S. M., Bieber, L. L., & Tolbert, N. E. (1978). A modification of the Lowry procedure to simplify protein determination in membrane and lipoprotein samples. *Analytical Biochemistry, 87*, 206–210.

Marmon, S. K., & Undeland, I. (2010). Protein isolation from gutted herring (Clupea harengus) using pH-shift processes. *Journal of Agricultural and Food Chemistry, 58*(19), 10480–10486.

Nolsøe, H., & Undeland, I. (2009). The acid and alkaline solubilization process for the isolation of muscle proteins: State of the art. *Food and Bioprocess Technology, 2*(1), 1–27. http://doi.org/10.1007/s11947-008-0088-4.

Panpipat, W., & Chaijan, M. (2017). Functional properties of pH-shifted protein isolates from bigeye snapper (*Priacanthus tayenus*) head by-product. *International Journal of Food Properties, 20*(3), 596–610.

Pires, C., Costa, S., Batista, A. P., Nunes, M. C., Raymundo, A., & Batista, I. (2012). Properties of protein powder prepared from Cape hake by-products. *Journal of Food Engineering, 108*(2), 268–275.

Rawdkuen, S., Sai-Ut, S., Khamsorn, 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.

Romero, A., Cordobés, F., Guerrero, A., & Puppo, M. C. (2014). Influence of protein concentration on the properties of crayfish protein isolated gels. *International Journal of Food Properties, 17*(2), 249–260.

Surasani, V. K. R., Tyagi, A., & Kudre, T. (2016). Recovery of proteins from rohu processing waste using pH shift method: Characterization of isolates. *Journal of Aquatic Food Product Technology, 26*(3), 356–365.

Taskaya, L., Chen, Y.-C., & Jaczynski, J. (2009b). Functional properties of proteins recovered from silver carp (Hypophthalmichthys molitrix) by isoelectric solubilization/precipitation. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology, 42*(6), 1082–1089.

Undeland, I., Hultin, H. O., & Richards, M. P. (2002a). Added triacylglycerols do not hasten hemoglobin-mediated lipid oxidation in washed minced cod muscle. *Journal of Agricultural and Food Chemistry, 50*(23), 6847–6853.

Undeland, I., Kelleher, S., & Hultin, H. (2002b). Recovery of functional proteins from herring (Clupea harengus) light muscle by an acid or alkaline solubilization process. *Journal of Agricultural,* 7371–7379 (Retrieved from).

Yongsawatdigul, J., & Park, J. W. (2004). Effects of alkali and acid solubilization on gelation characteristics of rockfish muscle proteins. *Journal of Food Science, 69*(7), 499–505.

Zhong, S., Liu, S., Cao, J., Chen, S., Wang, W., & Qin, X. (2016). Fish protein isolates recovered from silver carp (Hypophthalmichthys molitrix) by-products using alkaline pH solubilization and precipitation. *Journal of Aquatic Food Product Technology, 25*(3), 400–413.