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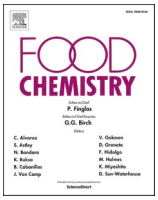

Physicochemical and functional properties of protein isolated from herring co-products; effects of catching season, pre-sorting, and co-product combination

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ABSTRACT

Effect of catching season (spring vs fall), pre-sorting and selective recombination of different herring filleting co-products on protein yield during valorisation using the alkaline pH-shift technology was studied. Impacts of the pre-processing conditions on lipid oxidation, rheological, structural and functional properties of the proteins were also investigated. The sorted frame fraction resulted in the highest protein yield, myosin content, gel-forming capacity and gel whiteness. pH-shift processing triggered severe proteolytic degradation and lipid oxidation in the head fraction imposing a low-quality protein isolate. The unsorted co-products and the combinations head + frame and head + frame + tail gave protein isolates with gelation and oxidative quality being better than head but below the isolate from frame alone. The spring co-products produced protein isolates with better overall quality than the fall co-products. Altogether, the results revealed the advantage of sorting herring co-products, and the influence of season on protein extraction from herring co-products.

1. Introduction

The worldwide demand for protein rich resources is increasing due to the growing and aging world population. Although fishing is considered as depletion of natural resources, aquatic foods can play a significant role to provide more healthy and sustainable future foods to the growing and aging population (Gephart et al., 2016; Hallström et al., 2019). Of the captured aquatic resources, pelagic fish such as herring generate lower Greenhouse Gas (GHG) emissions than all fed aquaculture (Gephart et al., 2021). However, side streams of herring filleting including heads, tails, frames and viscera encompassing over 50 % of the total fish weight currently goes to low-value products such as animal feed or, in worse cases, are even wasted. These co-products contain valuable proteins, polyunsaturated fatty acids (PUFA), essential amino acids, vitamins and minerals (Wisuthiphaet, Kongruang, & Chamcheun, n.d.; Wu, Forghani, Abdollahi, & Undeland, 2021). Increasing processing yield and efficiency in utilization these resources may further reduce environmental impact per kg of food produced from marine resources. Therefore, optimal utilization of fish co-products as part of the biomass which is unavoidably harvested and development of technologies enabling their value addition to high quality proteins is urgently needed.

Filleting co-products of small pelagic species such as herring are not currently sorted in the industry and are often combined as one fraction which limits their application potential for food production. In this mixture of co-products, blood, enzymes, lipids and microorganisms from fractions such as head and viscera can contaminate cleaner parts e.g., the frame. For example, proteolysis induced by proteases natively active in head and viscera can result in a substantial loss of protein functionality such as gelation and water holding capacity (Chen & Jaczynski, 2007). Combining the co-products could also increase the risk of lipid oxidation considering the high content of prooxidants such as heme and lipoxygenase (LOX) in some fractions as the head and high content of PUFA in viscera (Abdollahi, Marmon, Chaijan, & Undeland, 2016; Wu et al., 2021). For example, our recent study has shown that the very high amounts of haemoglobin (Hb) and LOX in herring head resulted in very quick lipid oxidation in this fraction while other fractions such as viscera or frame were more stable (Wu et al., 2021). Hinchcliffe et al. (2019) described a lower lipid content in protein extracted from head + frame and head + frame + viscera for feed application compared to from viscera alone, while a similar total protein yield for the mentioned co-product combinations was obtained (Hinchcliffe, Gunnar, Jönsson, & Sundell, 2019). In addition, bones and other unwanted materials as skin and connective tissue can make the protein isolation process more complicated and resource demanding. Therefore, proper sorting of

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herring and other fish co-products into separate fractions will be very important to enable successful value addition of these raw materials to protein isolates or other high value products. In addition, understanding the link between co-product combination and protein isolate quality will allow optimized use of the different co-product fractions.

The complex nature of fish co-products and their susceptibility to lipid oxidation have driven continuous research to find the most suitable protein recovery methods which also retain protein functionality and nutritional properties. The three main methods described are mechanical meat-bone separation (Froning et al., 1981; Abdollahi, Wu, & Undeland, 2021), enzymatic hydrolysis (Liaset, Lied, & Espe, 2000) and the pH shift process (Abdollahi et al., 2021). Among these technologies, pH-shift processing has shown good potential in extracting functional gel-forming proteins from a wide range of fish co-products, including herring, as shown by a series of studies from our group (Abdollahi et al. 2016, Abdollahi, Rezaei, Jafarpour, & Undeland, 2017; Abdollahi and Undeland 2018, 2019; Hinchcliffe, Gunnar, Jönsson, & Sundell, 2019) and other researchers (Chen, Tou, & Jaczynski, 2007; Freitas, Gautério, Rios, & Prentice, 2011; Panpipat & Chaijan, 2017). However, these studies have been limited to either a single co-product fraction e.g. frame (Abdollahi et al., 2021) or have been lacking focus on protein functionality or catching seasons (Hinchcliffe, Gunnar, Jönsson, & Sundell, 2019). Thus, there is a need for studies systematically evaluating the effect of seasonal origin, sorting and re-combining of different fish co-product fractions on protein isolation efficiency and functional properties of the isolated proteins targeting food applications.

The present study was aimed to investigate the effect of season (spring vs fall) and sorting of herring filleting co-products, without (head and frame), or with, selective re-combinations (head + frame or head + frame + tail) on protein isolation yield during pH-shift processing. In addition, the effects of these factors on rheology, structure, functionality, colour and lipid oxidation of the recovered protein isolates.

2. Materials and methods

2.1. Preparation of fish co-products

Fresh Atlantic herring (*Clupea harengus*) caught on 15 April 2020 and 21 October 2020 were subjected to filleting using a rebuilt filleting machine from Baader (Model Baader 36, Nordisher Maschinenbau Rudolf Baader Gmbh, Lubeck, Germany) placed at Sweden Pelagic AB (Ellös, Sweden). Herring filleting co-products were automatically sorted into four separate fractions including head, backbone (indicated as frame), viscera + belly flap together with small quantities of roe, milt or other organs (referred to as "viscera"), and tail. Unsorted herring co-products including a mixture of head, frame, tail and viscera were also collected as control. The five co-product fractions were covered with ice-filled plastic bags and transported within 3 h to the marine lab at Chalmers University of Technology. Upon arrival, sorted and unsorted herring fractions were immediately ground using a table-top meat grinder (C/E22 N, Minerva Omega group, Italy) equipped with a plate with 4.5 mm holes, and subsequently mixed to complete homogeneity. Thereafter the mince produced from the four individual fractions 'frame', 'head', 'tail' and 'viscera' as well as the mince from 'unsorted' co-products was frozen and stored at −80 °C until further usage.

2.2. Protein isolation using the pH-shift process

Mince from herring heads, herring frames, head + frame combined (1:1 ratio on w/w basis), head + frame + tail combined (1:1:1 ratio on w/w basis) and unsorted co-products from fall were subjected to pH-shift processing to evaluate how individual co-product fractions or selectively recombined fractions responded to this process. For spring, only mince from frame and unsorted co-products were processed to evaluate the effect of season.

Minces were subjected to alkaline pH shift processing following the main steps reported by Undeland, Kelleher, and Hultin (2002), with minor modifications. A portion of 100 or 500 g fish mince was homogenized with 6 parts of cold distilled water for 1 min at 8000 rpm on ice using a Silverson Homogenizer (LCM5 lab mixer, Silverson, USA). Then, the homogenate was adjusted to pH 11.5 using 2 M NaOH. The pH adjusted homogenate was incubated on ice for 10 min while stirring and then centrifuged at 8000 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 the floating lipid layer using a metal sieve and from sedimented insoluble residues containing skin and bones. The pH of the separated protein layer was then adjusted to pH 5.5 with 2 M HCl and incubated for 10 min at pH 5.5 on ice followed with a second centrifugation step at 8000×*g* (4 °C, 20 min) to dewater the precipitated proteins. If needed to reach a moisture content close to 80 %, the protein pellet was subjected to an additional centrifugation of 10 min at 8000×*g*. Cold NaOH (2 N) (~4 °C) was then used to adjust the pH of recovered protein isolates to 7 under cold conditions (<4 °C) and if required, the moisture content was adjusted to 80 % ± 1.5 % by distilled water addition. The recovered protein isolates were stored at −80 °C until used.

2.3. Protein solubility, precipitation and total yield during pH-shift processing

The protein solubility, precipitation and total yield during alkaline pH-shift processing were studied in triplicates by measuring the protein content of the initial homogenate at alkaline pH 11.5 (H), the first supernatant (S1) and the second supernatant (S2) using the Lowry method as modified by Markwell, Haas, Bieber, and Tolbert (1978). Calculations of solubilization yield, precipitation yield and total yields were performed with Eqs. (1)–(3).

$$\text{Solubilization yield}\,(\%) = \frac{\text{Protein content of S1 (mg)}}{\text{Protein content of H (mg)}} \times 100\% \quad (1)$$

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

$$\text{Total yield}\,(\%) = \frac{\text{Protein content of S1 (mg)} - \text{Protein content of S2 (mg)}}{\text{Protein content of H (mg)}} \times 100\% \quad (3)$$

2.4. Characterization of protein isolates

2.4.1. Proximate composition

The determination of moisture, protein, and lipid content of herring filleting co-products and the derived protein isolates was performed as described by Abdollahi et al. (2021). Protein content of the raw materials (n = 3) and their corresponding isolates (n = 2) was determined by subjecting 500 mg samples to a LECO nitrogen analyzer (TruMac-N, LECO Corp., St. Joseph, MI, USA) according to the Dumas method using a nitrogen-to-protein conversion factor of 5.58 (Mariotti & Tomé, 2008). The total lipid content of raw materials (n = 4) protein isolates (n = 2) was measured according to the method of Lee, Trevino, and Chaiyawat (1995) as modified by Undeland Kelleher & Hultin (2002). A chloroform:methanol ratio of 2:1 (v/v) was used for initial minced raw materials, while for the leaner protein isolates a 1:1 ratio was used. Moisture content of both raw materials (n = 3) and corresponding protein isolates (n = 2) was measured by calculating weight differences after drying the samples at 105 °C for 24 h.

2.4.2. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS-PAGE)

The polypeptide pattern of heads, frames and unsorted co-products from fall, as well as all the extracted protein isolates were investigated according to the method of Laemmli (Laemmli, 1970) as explained by Abdollahi & Undeland (2018) with potential modifications if needed.

2.4.3. Analysis of lipid oxidation

Peroxide value (PV) and thiobarbituric acid reactive substances (TBARS) were analysed to monitor the level of lipid oxidation products in sorted herring filleting co-products and their extracted protein isolates. Both of these analyses were conducted as described by Wu, Ghirmai, and Undeland (2020). The lipid extractions were performed in duplicates and duplicate PV and TBARS determinations were done on each of the emerging chloroform and methanol extracts, respectively.

2.4.4. Rheological characterization of protein isolates

Protein isolates from the different co-products were partially thawed in a tight plastic bag under running tap water until the core temperature reached 0 °C. Forty g isolate was then divided into small pieces and the moisture content of the samples was adjusted to 81.5 % by addition of ice-cold distilled water The sample was ground for 30 s in a small chopper on ice followed by 2 min chopping in the presence of 2 % w/w of NaCl to develop a homogeneous paste. Using a spatula, 1–2 g sample was loaded on a dynamic rheometer (Paar Physica Rheometer MCR 300, Anton Paar GmbH, Austria). Dynamic viscoelastic properties of the pH-shift produced protein isolate were identified using parallel-plate geometry (25 mm plate diameter and 1 mm plate gap) operated in an oscillating mode. The exposed sample perimeter was covered with inorganic oil to prevent evaporation. The samples were subjected to an *in situ* gelation at three steps in the rheometer. The storage modulus (G') over time was recorded when temperature increasing from 20 °C to 90 °C at the constant heating rate of 5 °C/min. Then the sample was subjected to an isothermal oscillation at 90 °C for 30 min, followed by ramping temperature down to 20 °C at a constant cooling rate of 5 °C/min. The test was done in a linear viscoelasticity region (1 % strain and 0.1 Hz frequency) of the samples.

2.5. Gel preparation and characterization

The residual of the paste prepared for rheology was stuffed into plastic tubes with a diameter of 15 mm. Both ends of the tubes were closed and sealed tightly and were subjected to a two-step heating: first 30 min in a 35 °C water bath followed by 20 min at 90 °C. 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.

2.5.1. Texture profile analysis of the gels

The texture profile analyses (TPA) test was used to measure textural properties (hardness, springiness, cohesiveness, chewiness) of the gels as explained by Abdollahi et al. (2017). The gels produced from the protein isolates were equilibrated to room temperature (23–25 °C) for 1 h. Cylinder-shaped gel samples with a length and height of 1.5 cm were prepared for each gel. 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.

2.5.2. Water holding capacity of the gels

The gels produced the day before were chopped into equally sized samples of which 2 g (X) was used to measure the water holding capacity (WHC) based on the gravimetrical method of Cardoso, Mendes, Vaz-Pires, and Nunes (2009). The gel sample was wrapped within two layers of pre weighed (Y) Filter Paper (Sigma Aldrich, Germany), and centrifuged at 3000×*g* for 10 min at 20 °C in a 50 ml centrifuge tube. After centrifugation, the filter papers were weighed again (Z) and WHC (%) was calculated by equation (4) and presented as percentage (Abdollahi & Undeland, 2019a).

$$\mathrm{WHC}\,(\%) = \frac{\mathrm{X}*\left(\frac{\mathrm{M}}{100}\right) - (\mathrm{Z} - \mathrm{Y})}{\mathrm{X} - \left(\frac{\mathrm{M}}{100}\right)} \times 100\% \quad (4)$$

In which M is the initial moisture of the gel (81.5 %).

2.5.3. Color measurement of the gels

The surface color of the gels made from the protein isolates was measured with a colorimeter (CR-400, Konica Minolta Sensing, Japan) as explained by Abdollahi et al. (Abdollahi & Undeland, 2018). The color of the samples was measured in the CIE L*a*b* color space by holding a probe directly against the bottom of a flat polystyrene plate containing the samples. The color parameters were recorded with 5 replicate readings of L*, a* and b* for each protein gel at different locations of the plates and data were used for calculation of whiteness using equation (2) (Abdollahi & Undeland, 2018):

$$\mathrm{Whiteness} = 100 - \sqrt{(100 - \mathrm{L})^2 + \mathrm{a}^2 + \mathrm{b}^2} \quad (5)$$

2.6. Statistical analysis

The statistical analysis was conducted with SPSS software (IBM SPSS Statistics Version 22, IBM Inc., Chicago, USA). The results were reported as mean ± standard deviation (SD) (n ≥ 2 as indicated in each section). One-way analysis of variance (ANOVA) and Duncan's multiple range test was used to compare means. The type of raw material and the capture season have been considered separately as factors in ANOVA. Differences with a probability value of $p < 0.05$ were considered significant.

3. Results and discussion

3.1. Protein solubility and yield during pH-shift processing of herring co-products

Factors determining protein yield in pH-shift processing are the solubility of the proteins at extreme pH, the sediment size formed during the first centrifugation and the protein solubility at the precipitation pH (Nolsøe & Undeland, 2009). As can be seen in Fig. 1, the individual type and specific recombination of herring co-products significantly ($p < 0.05$) affected on total protein yield from the pH-shift process. The maximum protein solubility yield (80 %), precipitation yield (92 %) and total yield (74 %) were observed during protein extraction from the isolated frame fraction (Fig. 1). This could be due to the higher amount of muscle residue present in this raw material which could be more efficiently extracted using the pH-shift process. A previous study has

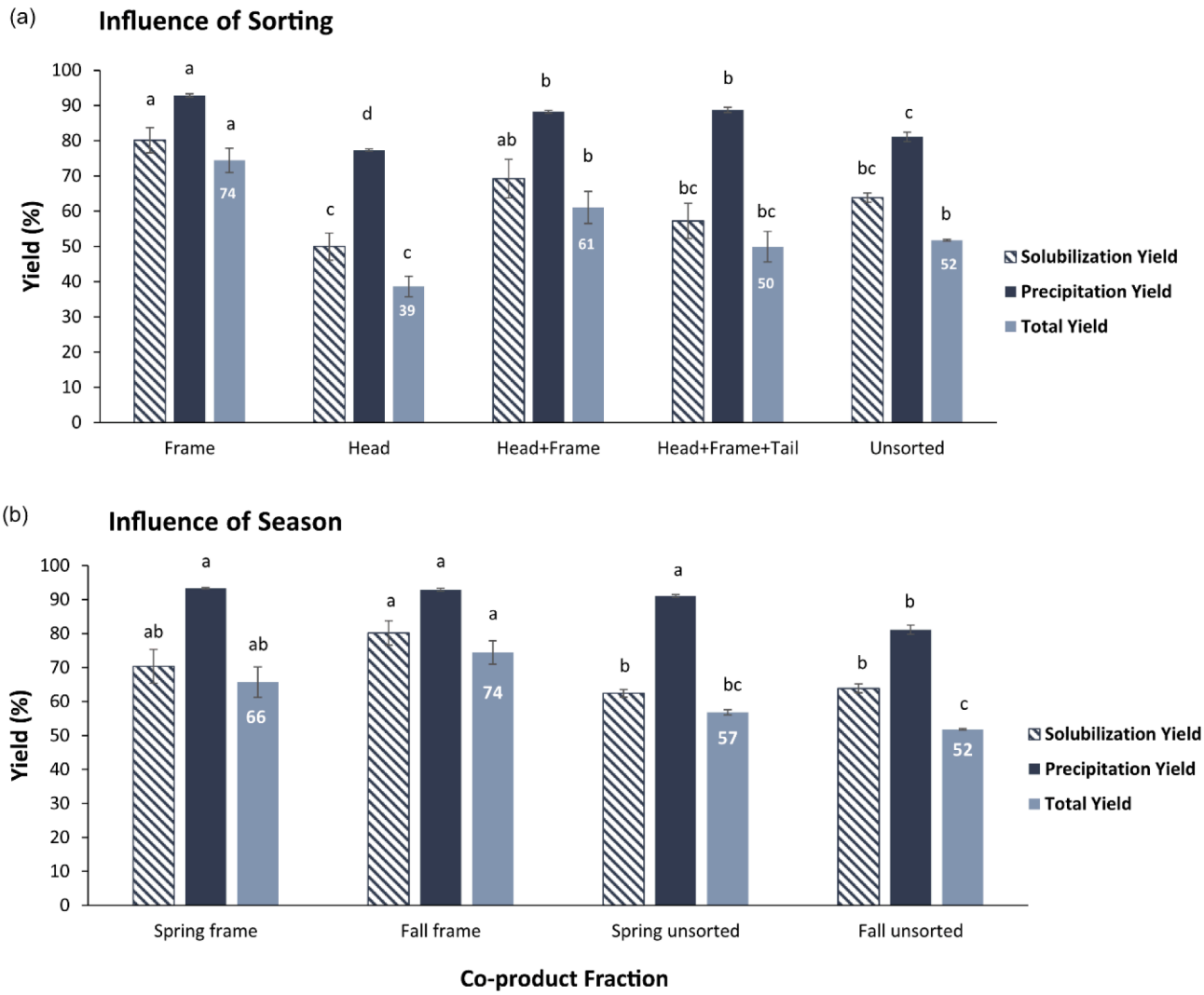

Fig. 1. Solubilization yield, precipitation yield and total yield during the pH-shift processing of unsorted, sorted and re-combined herring co-products from fall (a) and from unsorted co-products and frame caught during fall and spring (b). Different small letters show significance ($p < 0.05$) differences. Data are shown as mean values ($n = 3$) with error bars indicating standard deviation (STDEV.S).

shown that the muscle residue on herring frames contributes to up to 80 % of the frame weight (Abdollahi et al., 2021). The head fraction provided the lowest protein yield which was significantly ($p < 0.05$) lower than the protein yield from frame, head + frame + tail or unsorted co-products. This could be explained by the low amount of muscle residue in the head (see Supplementary Table 1) and the high ratio of bone and connective tissue (Hinchcliffe et al., 2019) Furthermore, the abundance of blood in the head, containing Hb, can result in fast rancidity development which can result in lower protein solubility due to lipid-protein interactions or protein crosslinking (Thorkelsson et al. 2008, Castell 1971, Wu et al. 2021). Also, >70 % of the heme pigments remain soluble in the second supernatant when using the alkaline version of the pH-shift process, meaning these proteins cannot be precipitated and recovered (Abdollahi et al., 2016). This could explain the low protein precipitation yield obtained for isolated heads compared with the other fractions (Wu et al., 2021). The combination of head + frame + tail showed comparable protein yield to the combination of only head + frame, which shows the limited effect of including tails in pH-shift processing, most likely due to its high ratio of bone and connective tissue. The unsorted co-products showed significantly ($p < 0.05$) lower protein precipitation yield than the combinations of head + frame, head + frame + tail or isolated frames. This could be related to the presence of viscera which contains high amounts of sarcoplasmic proteins that remain soluble at the isoelectric pH used for the precipitation (Hinchcliffe et al., 2019). However, the presence of viscera did not affect the total protein yield compared with the head + frame or head + frame-tail combinations, which is in line with results previously reported by Hinchliffe et al. (2019).

Catching season had no significant effect on total protein yield from frame alone or from unsorted co-products. However, the unsorted co-products from fall provided significantly ($p < 0.05$) lower protein precipitation yield compared with their spring counterpart. This could be due to the high amount of roe and milt in the unsorted spring co-products resulting in protein compositional differences between the two co-product batches, in turn affecting their solubilization pattern.

3.2. *Polypeptide pattern of the raw materials and protein isolates*

Polypeptide patterns of unsorted and sorted herring co-products from fall and all the proteins isolates are shown in Fig. 2. As can be seen, proteins isolated from frames showed much higher intensity of bands corresponding to myosin heavy chain (MHC) (~220 kDa) and actin (~45 kDa) compared with the unsorted co-products and proteins isolated from heads. This could reflect both the higher ratio of muscle proteins in the frame fraction, and the higher degree of protein hydrolysis happening in the minced unsorted co-products and head; both

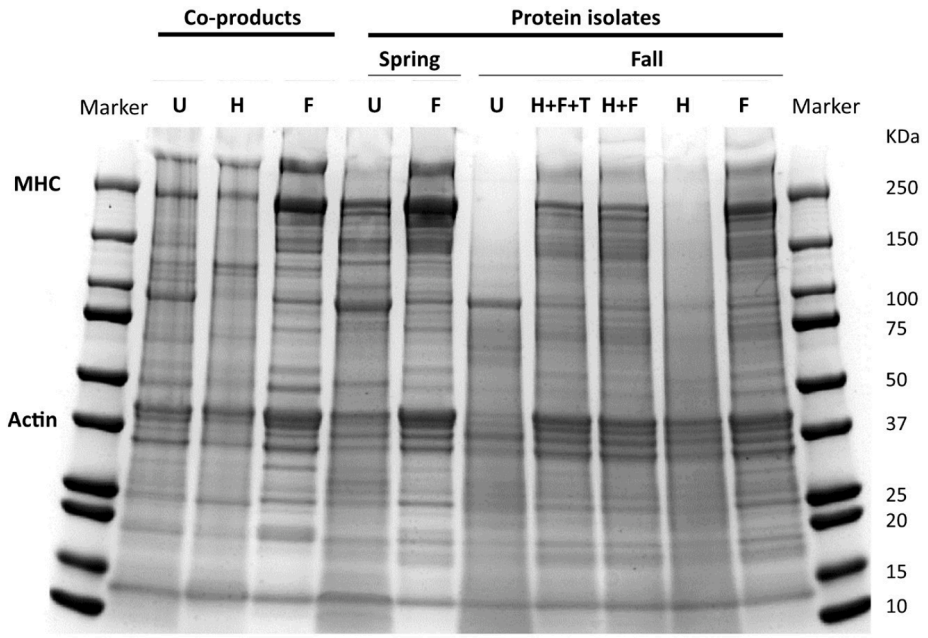

Fig. 2. Polypeptide pattern of unsorted and sorted herring co-products as well as protein isolates from unsorted, sorted and recombined herring co-products from two seasons. MHC: Myosin heavy chain, U: unsorted, H: head, F: frame, T: tail. 20 μg protein was loaded onto each lane.

carrying more enzymes. Protein isolates derived from frame from the two seasons also showed the highest band intensity for MHC and actin which substantially decreased when frame was mixed with head or head + tail. All MHC, and a large part of the actin, disappeared in protein isolates derived from head and unsorted co-products from fall. As seen for the raw materials, this was most probably due to the high activity of proteolytic enzymes naturally present in the viscera and head, creating proteolysis during pH-shift processing (Eble, Spragia, Ferguson, & Samarel, 1999). Previous studies have also reported partial degradation of myosin during pH-shift processing of fish co-products which could be due to activation of the proteolytic enzymes caused by the specific pH-cycle applied (Abdollahi & Undeland, 2019a; Marmon & Undeland, 2010). Since most proteases of herring are acidic (Undeland et al., 2002), the precipitation step is believed to be most critical. This also highlights the advantage of sorting out viscera and head before pH-shift processing of the co-products.

The spring protein isolates showed higher MHC concentrations than their respective fall samples, which corresponded with less MHC degradation also in the unsorted spring raw material. This could be probably due to a lower activity of digestive enzymes in herring during the spawning season compared to feeding season (Felberg et al., 2009). Overall, the polypeptide patterns showed that the type of co-product, their specific combination and catching season have a large impact on proteolysis during the pH-shift process. Thus, proper sorting of herring co-products can minimize the risk for protein degradation in some fractions.

Table 1
Peroxide value (PV) and thiobarbituric acid -reactive substances (TBARS) values in protein isolates and water holding capacity of the gels made of the isolates from unsorted, sorted and recombined herring co-products from two seasons.

Protein isolate	PV (μmol/kg)	TBARS (μmol/kg)	WHC (%)
Fall frame	436.65 ± 127.56^{cB}	27.24 ± 3.20^{dC}	74.10 ± 5.61aB
Fall head	2281.04 ± 275.83^{a}	217.69 ± 8.71^{a}	54.67 ± 2.69b
Fall head + frame	807.36 ± 287.77^{b}	74.97 ± 3.73^{c}	58.81 ± 0.69b
Fall head + frame + tail	1044.57 ± 198.31^{b}	100.25 ± 32.36^{b}	56.29 ± 0.45b
Fall unsorted	907.25 ± 197.75^{bA}	81.24 ± 5.50 ^{cA*}	61.38 ± 4.28bC
Spring frame	301.98 ± 197.75^{bA}	65.36 ± 6.36^{B}	66.75 ± 4.53BC
Spring unsorted	302.70 ± 105.20^{B}	53.68 ± 7.59^{B}	92.45 ± 1.02A

Values are means ± SD of 4 replicate determinations. Means with the same letter in each column are not significantly different ($p > 0.05$). Small letters are showing the differences related to the type of co-product or combination, capital letters show the differences between co-products from different seasons.

3.3. Lipid oxidation in herring co-product protein isolates

Primary (PV) and secondary (TBARS) lipid oxidation products were analysed in protein isolates from unsorted and sorted herring co-products from both fall and spring (Table 1). PV and TBARS values found in head protein isolates were significantly ($p < 0.05$) higher than in isolates from the other fall co-product combinations, while the lowest PV and TBARS values were found in isolates from the fall frame fraction. These findings are in line with previously reported PV and TBARS values of the respective non-processed herring raw materials after one day storage on ice (Wu et al., 2021). The high concentration of oxidation products in head protein isolates can be explained by the strong pro-oxidative capacity of Hb and lipoxygenase (LOX), which are present in high concentrations in the head (Maestre, Pazos, Iglesias, & Medina, 2009; Wu et al., 2021). The protein isolate from unsorted spring co-products was found to have a significantly ($p < 0.05$) lower PV compared to the protein isolate from unsorted fall co-products, which could be explained by a high tocopherol content in the spring raw material as recently observed by Wu et al. (2021). Tocopherol is known for its antioxidant capacity in both fish tissue and fish oil as found e.g. by Syväoja et al. (1985) and (Zuta, Simpson, Zhao, & Leclerc, 2007). Interestingly, the protein isolates from head + frame + tail showed significantly ($p < 0.05$) higher TBARS than isolates from head + frame from the same season. This was despite the fact that the initial PV and TBARS values previously found in tail raw material from fall and during their storage were lower than those in head (Wu et al., 2021). It is possible that the high Hb-level of the heads, when these contributed to 50 % of the raw material rather than 33 %, stimulated a very fast breakdown of lipid hydroperoxides into secondary products as aldehydes during the pH-shift process. The aldehydes could then react

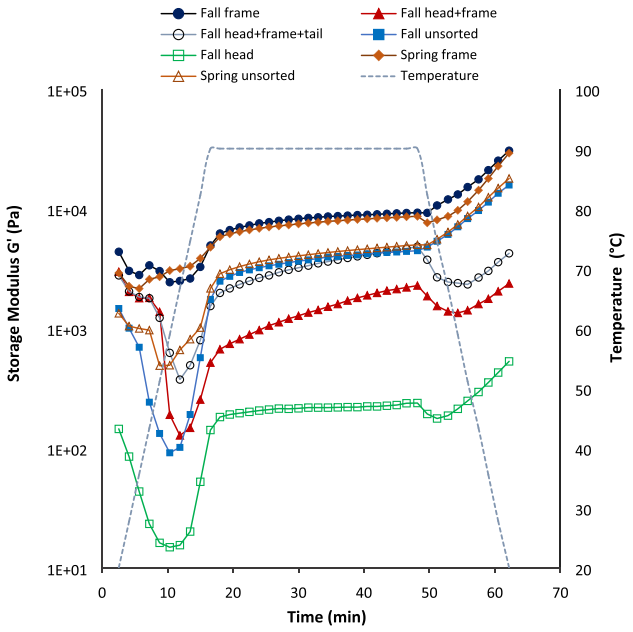

Fig. 3. Rheological behaviour (storage modulus') of protein isolates from unsorted, sorted and recombined herring co-products from two seasons during *in situ* gelation via temperature ramp test including an initial heating step (5 °C/min from 20 to 90 °C), followed by an isothermal step (90 °C, 30 min) and a final cooling step (rate: 5 °C/min from 90 to 20 °C).

further with proteins/peptides to tertiary oxidation products as Schiff bases, lowering the levels of both hydroperoxides and carbonyls responding in the PV and TBARS tests, respectively. Earlier studies have shown that with pre-oxidized raw materials, the level of oxidation products in isolates can be reduced compared to with raw materials of higher initial quality (Undeland et al. unpublished). Also, TBARS of herring protein isolates produced in absence of antioxidants decreased during subsequent storage while it increased in isolates produced with antioxidants (Undeland et al. 2005); most likely for the same reasons as discussed here, i.e. a dominance of tertiary product formation.

3.4. Rheological properties of herring co-product protein isolates

Viscoelastic properties of proteins isolated from herring co-products varying with respect to their combinations or seasons over the thermal gelation process are shown in Fig. 3. The change in rheological properties shown as storage modulus (G') at different steps during the thermal cycle applied was different depending on the type of co-product and their combination but not by catching season. The gelation process for all the proteins started with a reduction in G' which continued until the temperature reached around 60 °C. This softening and reduction in G' is related to the reduction of electrostatic interactions and hydrogen bonds due to the increase in mobility of the protein chains and thermal agitation which are both induced by heating (Felix, Romero, Rustad, & Guerrero, 2017a). This softening has also been related to the denaturation of myofibrillar proteins and oxidation of sulfhydryl groups as seen for different sources of fish proteins (Kim et al. 2005; Yoon, Gunasekaran, and Park 2004). Further increase in temperature from 60 to 90 °C resulted in a quick increase in G' in all the samples. This reflects network formation in the protein paste via partially denatured proteins either in the form of globular proteins or of helical rod segments of myosin which promote network formation through sulphide-bonds (Felix, Romero, Rustad, & Guerrero, 2017b; Westphalen, Briggs, & Lonergan, 2006). Evolution of viscoelastic properties and the level of increase in G' at this step was remarkably different among protein isolates from different co-products; with minimum structure formation and increase in G' in isolates from head and maximum in isolates from frame. The protein isolates from unsorted co-products and the selected combinations of co-products were placed in between the two sorted co-products with respect to G' increase. This was in line with remarkably higher amount of MHC observed in frame protein isolates which could successfully contribute to structure formation during the heating. On the other hand, the lower amount of MHC observed in protein isolates from head and mixed co-products can explain the lower structure formation capacity at these samples. During the isothermal stage of the gelation process, conducted at a constant temperature of 90 °C, the network development proceeded but with much slower kinetics for most of the samples except proteins from head + frame and head + frame + tail. Finally, during the cooling step, an increase was observed in G' of all the samples. However, it was preceded by a small reduction in G' for protein isolates from head or in mixtures of head with other co-products. In this step, it is mainly physical interactions such as hydrogen bonds and hydrophobic interactions which are formed and which increases mechanical moduli (G'). These interactions are very important in stabilization of the protein gel, but the hydrogen bonds also play an important role in immobilization of water into the protein networks which in turn can affect WHC of the gels. The final G' at the end of the gelation process clearly differentiated the samples, with the two protein isolates from frame derived from spring and fall co-products showing the highest mechanical moduli which was followed by isolates from the unsorted sample. Head protein isolates showed the lowest gel-forming capacity. It has been shown also previously that reduction in protein molecular weight as a result of proteolysis results in reduction of gelation properties (Felix et al., 2017b). However, some degree of proteolysis, and especially when targeting globular proteins may help gelation by promoting hydrophobicity via exposure of some buried hydrophobic groups, which might partially explain the good gel-formation of protein isolates from unsorted co-products (Jin et al., 2014).

3.5. Textural properties of gels

Textural properties of the gels made of herring protein isolates, including hardness, springiness, cohesiveness and chewiness are shown in Fig. 4 a-d. The results of the TPA test were in accordance with the storage modules obtained during *in situ* gelation of the different protein isolates. Among the fall samples, the gels from the frame isolate showed the highest hardness, cohesiveness and chewiness, which was 2–3-fold higher than the values for gels made of isolates from frame + head, frame + head + tail or unsorted co-products. The higher gel-forming capacity of the frame protein isolate could be due to its high MHC content and the low lipid content. The gel derived from head protein isolate had low gel forming capacity as described by the low springiness, hardness, cohesiveness and chewiness (Fig. 4). This might be related to the observed proteolytic enzyme activity (Fig. 2) (Yi Chen Chen & Jaczynski, 2007) or to the high amount of Hb found in head (Wu et al., 2021) and lipid in the head protein isolate. Chaijan, Benjakul, Visessanguan, & Faustman (2006) described the lack of gel forming capacity by sarcoplasmic proteins comprising heme, and also the interference of heme with actomyosin gel matrix formation, leading to a lower breaking force and more deformation. The poor gelation was also in line with the low structure formation capacity found in head protein isolates during the heating and cooling steps of *in situ* gelation. Protein isolates from the designed combinations of co-product fractions (frame + head or frame + head + tail) and the unsorted samples performed almost similarly for all the textural parameters and ranked in between the values for frame and head protein isolate-based gels. This implies that it is the presence of head in the co-product mixture rather than the enzyme-rich viscera which negatively affects the gel-forming capacity of protein isolates. This again highlights the importance of sorting the herring co-products to achieve an optimum quality during their subsequent valorisation.

Both spring samples showed a significantly ($p < 0.05$) higher

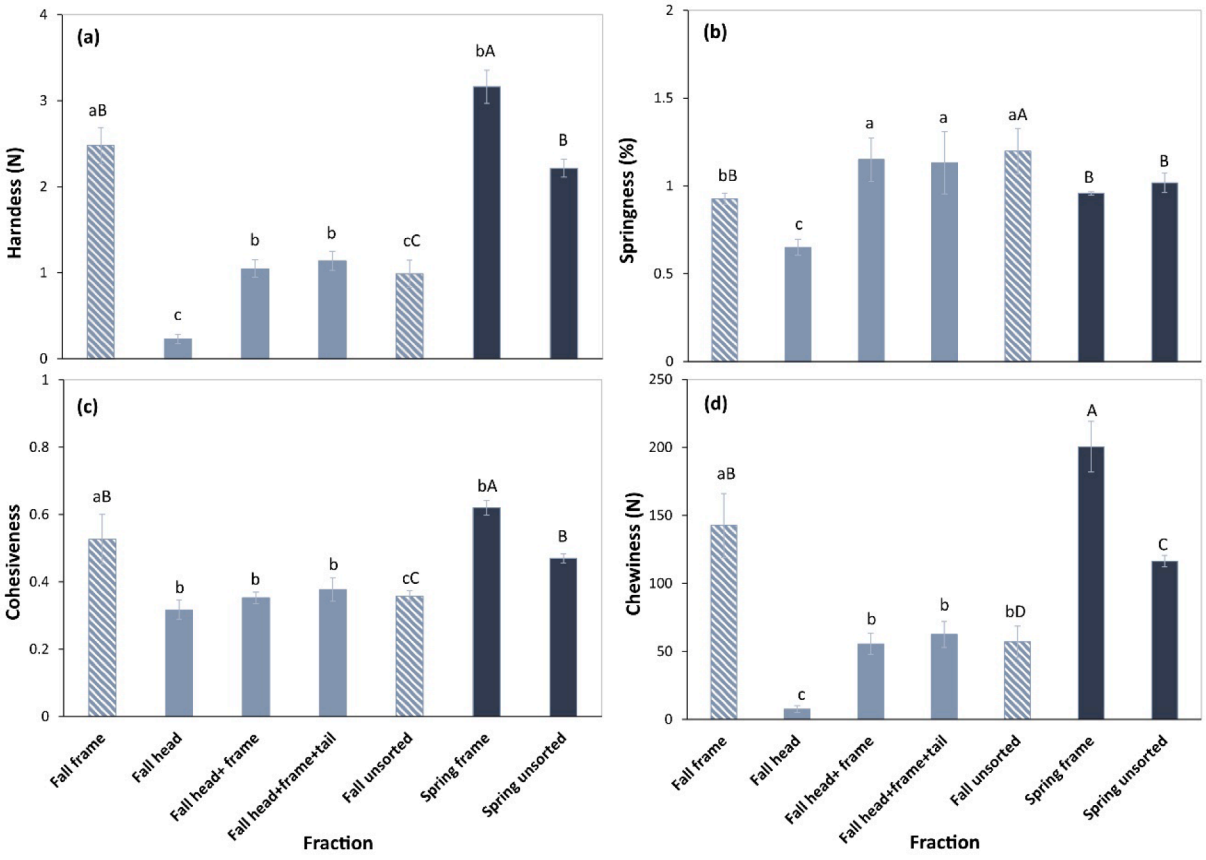

Fig. 4. Hardness (a), Springiness (b), Cohesiveness (c) and Chewiness (d)) of protein-based gels developed from proteins isolated from unsorted, sorted and combined herring co-products from two seasons. Values are means ± SD of 4 replicate determinations. Means with the same letter are not significantly different ($p > 0.05$). Small letters are showing the significant differences between different co-products and capital letters show the differences between co-products from different seasons.

hardness, cohesiveness and chewiness compared to the respective fall sample. The higher MHC content and the lower degree of proteolytic degradation in the frame and unsorted samples from spring as shown by the SDS-PAGE (see Fig. 2) might explain the better gel-forming capacity in these two samples. The finding is also in line with the storage modulus of these samples obtained during the *in-situ* gelation (Fig. 3). This means catching season of herring co-products can also affect functional properties of protein isolates recovered using the pH-shift technology, which is probably related to different enzymatic activity and lipid content in the co-products from herring caught at different seasons (Wu et al., 2021).

3.6. WHC of gels

The WHC of the herring protein-based gels was significantly ($p < 0.05$) affected by the type of co-products and their specific combination (Table 1). For the fall samples, the gel made of frame protein isolate showed a significantly ($p < 0.05$) higher WHC than the other samples. Gels made of head protein isolate, however, showed the lowest WHC among all the samples. This is very much in line with the textural properties of the gels (see Fig. 4). The higher WHC found in gels made of frame protein isolates could be related to their higher concentration of protein and MHC as well as the low concentration of lipids. These characteristics have probably resulted in better self-supporting gels with more homogeneous three-dimensional structure which better could hold water. As described earlier, a gel microstructure depends on the type of protein–protein interactions formed, the amount of sarcoplasmic proteins and lipids as well as the conformational changes of proteins (Chen et al., 2014; Bledsoe, Bledsoe, & Rasco, 2010; Intarasirisawat, Benjakul, Visessanguan, & Wu, 2014). In case of head, the high amounts of Hb and lipids as shown before (Wu et al., 2021) together with the observed proteolytic degradation of MHC and actin resulted in a gel which both was weak and had low WHC. On the other hand, the gel made of protein isolate from the unsorted spring co-products had a significantly higher WHC than the one from the unsorted fall sample. This could potentially

Table 2
Color properties of gels produced from different herring co-products from varying seasons.

Source of protein isolate	L*	a*	b*	Whiteness
Fall frame	51.17 ± 0.33^{aAB}	4.45 ± 0.05^{eB}	9.78 ± 0.01^{bB}	49.99 ± 0.33^{aA}
Fall head	38.08 ± 0.03^{d}	5.89 ± 0.04^{a}	6.50 ± 0.02^{c}	37.47 ± 0.05^{e}
Fall head + frame	44.03 ± 0.21^{c}	5.09 ± 0.01^{c}	8.57 ± 0.05^{b}	43.19 ± 0.15^{d}
Fall head + frame + tail	47.88 ± 0.05^{b}	4.75 ± 0.04^{d}	10.04 ± 0.11^{b}	46.70 ± 0.00^{b}
Fall unsorted	47.73 ± 0.19^{bC}	5.30 ± 0.06^{bA}	11.92 ± 1.35^{aA}	46.11 ± 0.13^{cB}
Spring frame	50.98 ± 0.30^{B}	3.59 ± 0.03^{D}	10.11 ± 0.06^{AB}	49.83 ± 0.22^{A}
Spring unsorted	52.07 ± 0.46^{A}	4.01 ± 0.00^{C}	11.73 ± 0.10^{A}	50.48 ± 0.47^{A}

Values are means ± SD of 5 replicate determinations. Means with the same letter in each row are not significantly different ($p > 0.05$). Small letters are showing the differences between different co-products, capital letters show the differences between co-products from different seasons.

be due to the higher degree of proteolysis in the fall protein isolates than in the corresponding spring isolates as it was found in their SDS-PAGE results (see Fig. 2).

3.7. Surface color of the gels

The measured color parameters and the whiteness of gels made from all herring co-products are summarised in Table 2. The whiteness of the gels from individual co-products and the selected combinations was close to the range previously reported for gels made of protein isolate from herring head, frame and tail (Abdollahi & Undeland, 2019a). Isolate from the head fraction showed a significantly ($p < 0.05$) lower whiteness than the other isolates (37.47), which could be possibly related to the high concentration of heme pigment remaining in the protein isolate as shown before (Abdollahi & Undeland, 2019b; Wu et al., 2021). These pigments can be oxidized >pH 11.5 or during isoelectric precipitation at pH 5.5 to yield brown metHb or metmyoglobin (metMb), which results in a lower a lower whiteness value (Abdollahi et al., 2016). Gels made of protein isolates from the fall frame fraction showed significantly ($p < 0.05$) higher whiteness than the other fall samples which was probably due to a low heme concentration in this fraction (Wu et al., 2021). Isolates from the head-frame + tail combination and the unsorted co-products had very similar whiteness values, confirming it is the heme-rich head which mostly affects the whiteness of the samples. The gels made of frame protein isolates of both seasons had similar whiteness values, however, the gel made from unsorted spring protein isolate was found to be significantly ($p < 0.05$) whiter than its counterpart from fall. This is not in line with the reported Hb content of the mixed co-products, as reported by Wu et al. (2021) which might be related to the higher fat content in the spring isolate (see Supplementary Table 1), which could result in higher whiteness due to increased light scattering from the emulsion created when oil is comminuted with fish muscle proteins and water (Chen & Jaczynski, 2007).

4. Conclusions

Protein isolation yield from herring co-products as well as the molecular weight distribution and functional properties of the recovered proteins were found to be strongly dependent on the type(s) of herring co-product processed and the catching season. The frame fraction provided the highest protein yield and its protein isolate had the highest MHC concentration, resulting in the best gel- forming capacity. The gels from frame also had the highest whiteness. The head fraction resulted in the lowest protein yield and its protein isolates showed the least gel-forming capacity, likely because of the high proteolytic activity in this fraction. Gels of head isolates also were the least white, most likely due to the heme- and melanin-rich nature of heads. The combinations head + frame, head + frame + tail and the unsorted co-products showed comparable results for all parameters, performing better than head but worse than frame. Herring frame alone was found to be most promising co-product for protein extraction with the alkaline pH-shift process, but by removing the head fraction from the co-product mixture, also other combinations can be of interest. The tail and viscera fraction were found to have less negative impact on the color, gel-forming capacity and protein yield than the head fraction. However, the protein isolates of unsorted co-products showed much more proteolysis during pH-shift processing than the other combinations, most likely due to the presence of viscera.

Besides the type of co-product, the catching season affected the pH-shift processing of herring co-products. The co-products from spring were more difficult to process but resulted in protein isolates with higher MHC content and stronger gels. Protein isolates from unsorted spring co-products resulted in gels with a better color, lower lipid concentrations and a very high WHC compared to its respective fall sample.

Altogether, this research shows the importance of sorting herring co-products to allow their individual processing, or well-designed combinations. Also, it illustrated the potential need for adapting the pH-shift process conditions along with seasonal differences in the herring raw material throughout the year, thereby increasing the potential of using this sustainable protein source for industrial food applications.

CRediT authorship contribution statement

Eline van Berlo: Data curation, Investigation, Methodology, Visualization, Writing – original draft. **Ingrid Undeland:** Conceptualization, Writing – review & editing, Funding acquisition, Project administration. **Mehdi Abdollahi:** Supervision, Conceptualization, Writing – review & editing, Visualization, Funding acquisition, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2022.133947.

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