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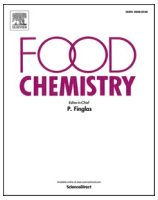
Food Chemistry 484 (2025) 144403

Contents lists available at ScienceDirect

Food Chemistry

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



Biomass recovery from herring brines: Exploring quality and functionality of protein and n-3 polyunsaturated fatty acids

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ARTICLE INFO

Keywords:
Clupea harengus
Fish
Seafood
Marination brine
Protein
Omega-3 polyunsaturated fatty acids
Volatile compounds
Circular economy
Emulsion property

ABSTRACT

Protein-enriched biomasses recovered from herring brines using dissolved air flotation (DAF) at native or low pH were studied in terms of nutrients, volatile compounds, emulsion properties, foaming properties and protein structure. Dried biomasses from native or acidified 3 % pre-salting brine (SB and SB-A) and spice brine (SP and SP-A) contained 69–72 % and 20–31 % protein, respectively; 7.8–10.2 % and 9–18 % lipids, respectively, and 4.4–7.5 % and 33–36 % ash, respectively. Of total fatty acids, up to 44 % were long-chain monounsaturated fatty acids (LC MUFA) and 17 % LC n-3 polyunsaturated fatty acids (PUFA). Both emulsion activity index (EAI) and foaming capacity (FC) were higher for SP/SP-A than SB/SB-A. In both cases, there was a minimum at pH 5. The findings suggest that currently wasted herring brine side streams hold promise for production of sustainable, functional protein ingredients which can provide both flavor and nutrients.

1. Introduction

Herring processing in northern Europe annually comprises approximately one million tonnes of landed raw material, leading to significant volumes of process waters being generated at various stages of the production, such as pre-processing storage, filleting, pre-salting, and acid/salt/spice marination (Baron et al., 2015). These waters are rich in macro- and micronutrients like proteins, peptides, long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFA) and minerals (Osman et al., 2015; Stefánsson and Nielsen, 1995; Szymczak and Kołakowski, 2012). Regarding proteins, there are both salt-soluble myofibrillar proteins and sarcoplasmic proteins like cathepsins and other enzymes (Szymczak and Kołakowski, 2012); (Osman et al., 2015) Additionally, the waters emerging during herring marination also harbor compounds from the marination formulation per se, including polyphenols from spices, salt and acetic acid (Gringer et al., 2016; Szymczak and Kołakowski, 2012). Given the many steps included in the production of marinated herring when going from the vessel to final glass jar, it has been estimated that around 7 m3 water is consumed per ton final herring product (Baron et al., 2015). Through leaching into pre-salting and marination brines, our previous data (Forghani et al., 2023) indicate that 10 kg pure proteins and 2 kg pure fatty acids are lost per tonne of fresh herring entering into processing. Given an annual processing of 10,000 t herring; 100 t proteins and 20 t fatty acids are thus lost as aqueous side streams. Today, the created aqueous side streams become waste waters, which are double costs for herring companies since they in many countries must pay for in house pre-cleaning, while they at the same time loose valuable herring-derived nutrients. To better align with the United Nations Sustainable Development Goals (SDGs), particularly No. 2, 3, 12 and 14, Zero Hunger, Good Health and Wellbeing, Responsible Consumption and Production, and Life Below Water, aqueous side streams generated during seafood processing should thus be treated in a food grade manner rather than as waste, so that the recovered materials can be maintained in the food chain. This would however require that cost-efficient food grade recovery techniques are available for seafood processors to implement. Dissolved air flotation (DAF), already in place in most seafood processing companies to reduce the BOD of waste waters, is a cost-effective separation technique based upon the incorporation of air bubbles to adsorb molecules which are dissolved into the water. In order to maximize the BOD reduction, a wide range of salts, e.g., based on iron

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https://doi.org/10.1016/j.foodchem.2025.144403
Received 19 November 2024; Received in revised form 6 April 2025; Accepted 17 April 2025
Available online 18 April 2025

or aluminum, or synthetic molecules such as polyacrylamide are normally used prior to DAF to enhance the particle size and improve the floatability of dissolved compounds (Mavros and Matis, 2013). Examples of their application within seafood processing comprises the use of polyacrylamide to remove protein from surimi wash waters (Park, 2005), coagulation of scallop processing water with $FeCl_3$ (Krofta et al., 1988), and coagulation/flocculation of fish processing waters using a combination of Tanfloc, $FeCl_3$ and Zetag, the latter providing a 92 % COD reduction (Karnena et al., 2022). The mentioned treatments, however, makes the removed sludge unfit for any higher value purposes as feed or food (Park, 2005) (Genovese and González, 1994) (Genovese and González, 1994). To open for such applications, alternatives to DAF must be used, or the DAF pre-treatment must be done with a food/feed grade coagulant/flocculant. Regarding the former, pressure-driven membranes as microfiltration, ultrafiltration, nanofiltration and reverse osmosis have for instance been used to recover an astaxanthin-rich-fraction from shrimp cooking water (Amado et al., 2016) as well as to recover proteins from poultry processing water (Białas et al., 2015), soy protein isolation process water (Cassini et al., 2010), cheddar cheese effluents (Adams et al., 2013) and herring process waters (Lee et al., 1996; Mariotti and Tomé, 2008; Osman et al., 2015). Membrane processes do not require pre-treatments with flocculants/coagulants, but inevitably entail fouling, especially with lipid-rich waters (Osman et al., 2015), which is a major obstacle to reach efficiency in the separation (Hube et al., 2020). DAF does not present this challenge, and additionally, is particularly effective in handling high-lipid aqueous streams, making it a suitable choice for herring-processing waters.

Previous research has successfully explored the recovery of nutrients from shrimp and herring process waters using polysaccharide or acid-driven flocculation and DAF (F-DAF), resulting in the recovery of 13 % to 97 % protein depending on the flocculation method To successfully introduce new protein-enriched biomasses recovered from herring brines and other aqueous seafood side streams into food products, more knowledge is however needed on their quality, including their nutrient composition, techno-functionality and volatile compound/flavor profile. To date, very limited work is done within this area, mainly addressing gelling property, emulsification and foaming of fractionated spice brine (Forghani et al., 2023; Taheri et al., 2014).

This study's objective was to investigate the compositional, structural and techno-functional properties of biomass recovered from two types of herring brines using DAF, with or without acid-driven pre-flocculation. Among compositional features, polypeptide profiles, essential amino acid content and fatty acid composition were investigated. Further, volatile profiles, emulsion, and foaming properties as well as protein structural features were studied to unravel the applicability in various food products. Overall, this research contributes significantly to new pathways stimulating more efficient resource utilization and reduced environmental impact in seafood processing.

2. Material and methods

2.1. Material

Herring brines generated during pre-salting of herring in 3 % NaCl and during subsequent marination of herring in spice brine were obtained from Sweden Pelagic AB in Ellös, Sweden, and from Klädesholmen Seafood AB in Rönnäng, Sweden, respectively. Hydrochloric acid (30 %), used for acidification prior to F-DAF was sourced from Nitor, Sweden.

2.2. Recovery of biomasses

In March 2018, on-site DAF trials were conducted at the respective companies that generated 3 % pre-salting brine and spice brine. A pilot size flotation unit from Bio-Aqua A/S, Denmark, was used for the DAF (Forghani et al., 2020). For the 3 % pre-salting brine, two DAF runs were performed: one as a control run with 3 % pre-salting brine at its native pH (6.5), referred to as SB, and another with the brine acidified to pH 4.7, referred to as SB-A. Similarly, for the spice brine, two DAF runs were conducted: one control run with spice brine at its native pH (5.8), referred to as SP, and another run where the spice was acidified in two steps to pH 4.2, referred to as SP-A. First it was brought to pH 4.5 using another herring side stream; vinegar brine, and then to pH 4.2 using 1 N HCl. The treated brines were pumped at a rate of 300 L/h into the flotation unit (Forghani et al., 2020) while micro air bubbles were generated and injected into the incoming water. The generation of microbubbles continued for 20 min after which the inlet pumping ceased. Biomass collection commenced once a thick foam layer formed on top of the water in the flotation unit and continued regularly until the end of the flotation period (Fig. 1). The temperature of the process waters remained below 10 °C during the entire DAF treatment. Recovered biomasses were frozen stored at —80C, and then freeze dried in a Labconco freeze dryer.

2.3. Analytical methods

2.3.1. Proximate composition

The protein content of the freeze-dried biomasses was analyzed using a nitrogen analyzer (LECO TruMac N, MI, USA) with a conversion factor of 5.58 (19). Lipid content was determined gravimetrically following the method outlined by (Lee et al., 1996). Extraction was performed using chloroform:methanol (2:1). After vortexing for 10 s, 0.5 % NaCl was added to reach a ratio of 1:2.75 (*V*/V). Following phase separation, chloroform was recovered and evaporated at 40 °C. Dry matter and ash contents were measured gravimetrically by exposing the samples at 105 °C and 550 °C, respectively, for 24 and 3 h.

2.3.2. Polypeptide profiling of biomasses using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The polypeptide patterns of the freeze-dried biomasses were visualized using SDS-PAGE, following the method of Laemmli (Laemmli, 1970) employing precast mini linear gels (4–20 %, Bio-Rad, USA), as described by Forghani, Sørensen (Forghani et al., 2020). Protein bands were visualized by staining with Coomassie Brilliant Blue G-250.

2.3.3. Amino acid analysis

The freeze-dried biomasses were mixed with 4 mL of 6 N HCl and flushed with nitrogen gas for 30 s. Hydrolysis was then carried out by maintaining the tubes at 110 °C for 24 h. Subsequently, the hydrolyzed samples were filtered and diluted before amino acid determination using LC/APCI-MS, following the method outlined by Özcan and Şenyuva (2006), as described by Forghani et al. (2020). The peaks were compared against an amino acid standard mix (ref# NCI0180. 20,088, Thermo Scientific Pierce, USA). Results were reported as mg amino acid/g dried biomass ($n = 2$).

2.3.4. Fatty acid analysis

Fatty acid analysis of the freeze-dried biomasses was conducted after the described lipid extraction (Lee et al., 1996) and subsequent methylation with a modified version of Lepage and Roy (Lepage and Roy, 1986). C:17 as an internal standard was added at start of the lipid extraction. Methylation was carried out by adding 2 mL of toluene and 2 mL of acetylchloride:methanol (10 %), and the solution was incubated at 60 °C for 120 min. One mL of Milli-Q water and 2 mL of petroleum ether were then added to the tubes, which were vortexed for 10 s and centrifuged at 2500 ×*g* for 5 min. The upper phase was transferred to new tubes and evaporated under nitrogen gas at 40 °C. Evaporated samples were then dissolved in 0.5 mL isooctane. Identification and quantification of fatty acids were performed by GS-MS using an Agilent Technologies 7890 A GC system connected to Agilent Technologies 5975 inert Mass Selective Detectors (MSD) (Kista, Sweden), as described elsewhere (Cavonius et al., 2014). Total fatty acids were calculated as

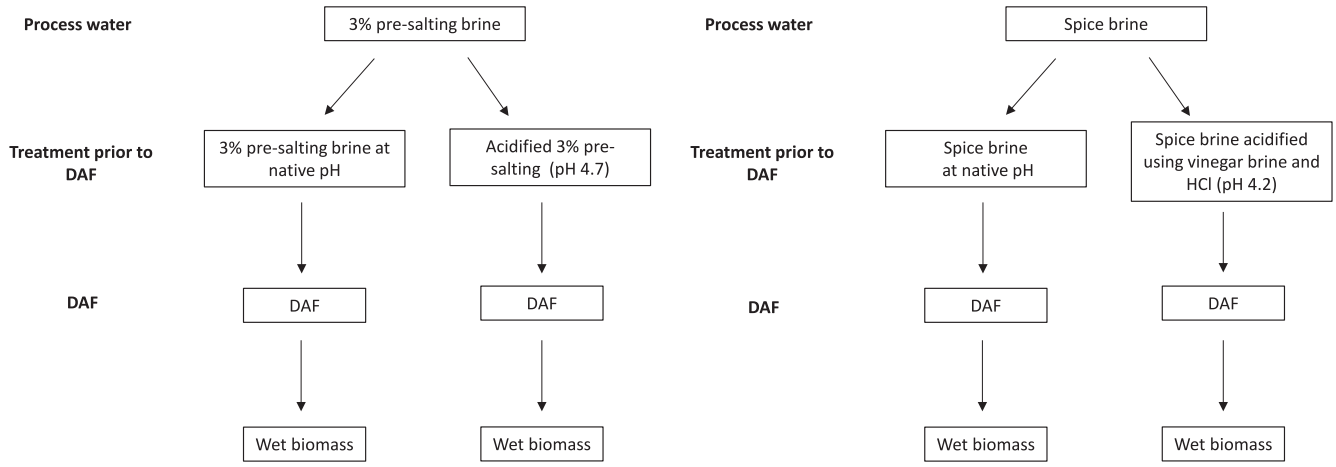

Fig. 1. Schematic figure of the applied biomass-recovery design, using DAF.

the sum of all measured fatty acids in the sample, minus the internal standard.

2.3.5. Volatile compounds of recovered and freeze-dried biomasses

The collection of volatile compounds was carried out using dynamic headspace 'purge and trap.' Volatiles from the freeze-dried biomass suspended in water were purged (37 °C) with nitrogen (260 mL/min) for 30 min and trapped on Tenax tubes. The trapped volatiles were desorbed and separated on GC (Agilent Technologies 6890 N, CA, USA) with a DB1701 column (30 m; i.d. 0.25 mm; 1 μm film thickness; Agilent Technologies). The oven program had an initial temperature of 45 °C for 5 min, followed by a gradual increase with 1.5 °C/min until 55 °C, 2 °C/min until 90 °C, and 8 °C/min until 230 °C, where the temperature was held for 8 min. The individual volatiles were analyzed by MS (Agilent 5973 Network Mass Selective Detector, Agilent Technologies; electron ionization mode, 70 eV; *m/z* scan between 30 and 250) and identified using the MS-library, with quantification performed through calibration curves of external standards. The results were reported as ng/g dried biomass ($n = 3$). The limit of detection (LOD) was determined to be 5 ng/mL.

2.3.6. Fourier Transform infrared (FT-IR) analyses of freeze-dried biomasses

FT-IR spectroscopic analysis was conducted on the dried biomasses following the procedure outlined by Abdollahi, Rezaei (Abdollahi et al., 2017). The spectra were generated using a Nicolet 6700 spectrophotometer (Thermo Scientific, MA, USA), by placing both the freeze-dried herring protein biomass and reference (deionized water) samples onto a crystal cell and scanning in the range from 4000 to 400 cm − 1.

2.3.7. Emulsion activity index, emulsion stability index, foaming capacity and foam stability of freeze-dried biomasses

The freeze dried biomasses' emulsion activity index (EAI) and emulsion stability index (ESI) were assessed following the protocol outlined by Ogunwolu, Henshaw (Ogunwolu et al., 2009). Initially, 300 mg of protein biomass was dispersed in 30 mL of distilled water, adjusting the pH to 3, 5, 7, 9, and 11. An oil-in-water emulsion was then prepared by combining the protein solution with 10 ml of sunflower vegetable oil and homogenizing at a speed of 20,000 ×g for 1 min. Fifty μl of the emulsion was the pipetted from the container's base; both immediately and after 10 min, into 5 ml of a 1 % SDS solution, followed by 20 s of vortexing. The absorbance of the solution was measured at 500 nm using a spectrophotometer, and the EAI and ESI values were calculated using the subsequent equations.

$$\text{EAI}\ (\text{m2/g}) = \frac{2 \times 2.303 \times \text{A0} \times DF}{C \times \varphi \times \theta \times 10000.}$$

where A0 = measured absorbance at 500 nm; DF = dilution factor = 200; θ = path length of the cuvette = 1 cm; C = the initial concentration of protein (g/mL);

φ = the volume fraction of oil in the emulsion = 0.25.

$$\text{ESI}\ (\text{min}) = \frac{\text{A10} \times \Delta t}{\Delta \text{A}}$$

where A10 is the absorbance at 10 min after homogenization;

Δt = 10 min; and ΔA = A0 − A10.

To measure foaming capacity and foaming stability of the protein-rich biomass, 250 mg of each powder was dispersed in 25 ml of distilled water (V-initial), and its pH was adjusted to 3, 5, 7, and 11. The dispersion was then homogenized using a Polytron Homogenizer (IKA, Brazil) for 2 min at 10,000 rpm. The volume of the formed foam immediately after homogenization (V1) and after 60 min (V60) was measured, and foaming capacity and foaming stability were calculated as follows:

$$\text{Foaming capacity}\ (\%)\ \frac{\text{V1} - \text{Vinitial}}{\text{Vinitial}} \times 100$$

2.4. Statistical analysis

One-way analysis of variance (ANOVA) and the Tukey test were used to determine significant differences between the studied variables; e.g. brine, type, recovery pH, biomass solubilization pH. Differences with a probability value of <0.05 were considered significant, and all data were reported in the form of mean ± SD. All experiments were run in triplicate ($n = 3$), except for dry matter and volatile compounds ($n = 2$).

3. Results and discussion

3.1. Characteristics of freeze-dried herring brine powders

The crude composition of freeze-dried herring brine biomasses is reported in Table 1. Biomass recovered from 3 % pre-salting brine (SB and SB-A) contained significantly higher ($p < 0.05$) protein (69 % and 72 %) as compared to those from spice marination brine (SP and SP-A) (21 % and 31 %). The ash content measured in SB and SB-A were found to be significantly lower compared to those of in SP and SP-A, reflecting the lower salt content present in the initial SB (1.8 %) as compared to SP (12.7 %). For both types of brines, biomasses recovered at native pH had significantly higher lipid content ($p < 0.05$) than those recovered at

Table 1
Proximate composition (%) of freeze-dried biomasses recovered with/without acidification and flotation of SB and SP.

Biomass	Protein content (%)	Lipid content (%)	Moisture content (%)	Ash content (%)
3 % pre-salting brine – SB	72 ± 0.4 ^{A}	10.2 ± 0.1^{B}	4.0 ± 0.3 ^{A}	4.4 ± 0.2^{D}
Acidified 3 % pre-salting - SB-A	69 ± 0.2 ^{A}	7.8 ± 1.2^{C}	3.1 ± 0.2^{B}	7.5 ± 0.1^{C}
Spice brine - SP	20 ± 0.0^{C}	14.0 ± 0.4 ^{A}	0.7 ± 0.2^{D}	33 ± 0.1^{B}
Spice brine acidified using vinegar brine and HCl - SP-A	31 ± 0.7^{B}	9.8 ± 0.7^{B}	2.0 ± 0.0^{C}	36 ± 0.0 ^{A}

SB, biomass from 3 % brine; SB-A, biomass from 3 % brine at pH 4.7; SP, biomass from spice brine; SP-A, biomass from spice at pH 4.2 using HCl and vinegar brine. Means with different superscript letters within the same column for each water type indicate significant differences among these samples ($p < 0.05$).

reduced pH; 10.2 and 14 % dw versus 7.8 and 9.8 % dw. We previously reported that biomasses recovered from 5 % pre-salting herring brine at both native pH and pH 4.7 contained 36 % and 38 % protein (dw) and 31 % and 28 % lipids (dw), respectively (Forghani et al., 2023). However, in the present study, the 3 % SB was generated in March while the previously used 5 % SB was from F-DAF recovery tests performed in October (Forghani et al., 2023). Jensen, Jacobsen (Jensen et al., 2007) reported that herring lipid content is at peak during July–August (15.6–16.9 %) and then gradually decreases from September to May (10.1–4.5 %). Other studies have shown minimum lipids in Feb-March, and maximum in Sept-Oct (Aidos et al., 2002a). It is also known that the lipids are more loosely bound in the herring tissue in the early fall when the herring gains fat, compared to in the spring (M. Kuhlin, pers. comm). This thus indicates that higher lipid content in the biomasses from 5 % pre-salting brine likely results from the higher lipid content of the used herring. Further, the crude 3 % SB contained 1.0–1.1 % protein (ww) and 2.9–3.3 % dry matter, respectively, which was less than the corresponding values in the earlier used 5 % SB (1.8–2.0 % protein (ww) and 5.6–5.8 % dry matter). The lower amount of solids in the presently used 3 % SB could positively affect the protein recovery via F-DAF (Forghani et al., 2023) and subsequently lead to higher protein content in the biomasses.

The polypeptide profiling of biomasses recovered from SB and SB-A revealed distinct bands between 13 to >250 kDa (Fig. 2). In SB, bands were observed at 13, ~19, 26, 30, 33, 35, 37, 40, 73, 90, 138, and 213 kDa. Both contractile proteins (e.g. myosin light chains, actin, troponins, and myosin heavy chain) and sarcoplasmic proteins (e.g. albumin) were present (Osman et al., 2015). Notably, SB-A exhibited additional bands at 27, 47, 53 and 65 kDa which was likely attributed to precipitation induced by the pH adjustment carried out during the DAF treatment. And as expected, the acidification also improved the recovery of specific polypeptides, such as those at 13, 26, 33, and 40 kDa, likely due to their isoelectric precipitation. However, the intensity of the 213 kDa band decreased, which may be explained by the relative increase in the content of other polypeptides.

This increased the visualization of bands below 50 kDa and omitted bands above 50 kDa (Forghani et al., 2023). As for SB, the pH adjustments during the DAF technique influenced solubility and subsequent recovery of SP-proteins. In SP-A, there was an enhancement in the intensity of the band at 35 kDa. Additionally, new bands appeared at 25, 27, 34, 37, and 48 kDa compared to SP, which only displayed bands at 13, 35, and 40 kDa. The notable differences in the molecular weight of polypeptides between biomasses originating from SB and SP can be attributed to the prolonged marination period and extended proteolysis of the latter.

The results highlight the dynamic nature of protein interactions during the recovery process, particularly under the acidic conditions

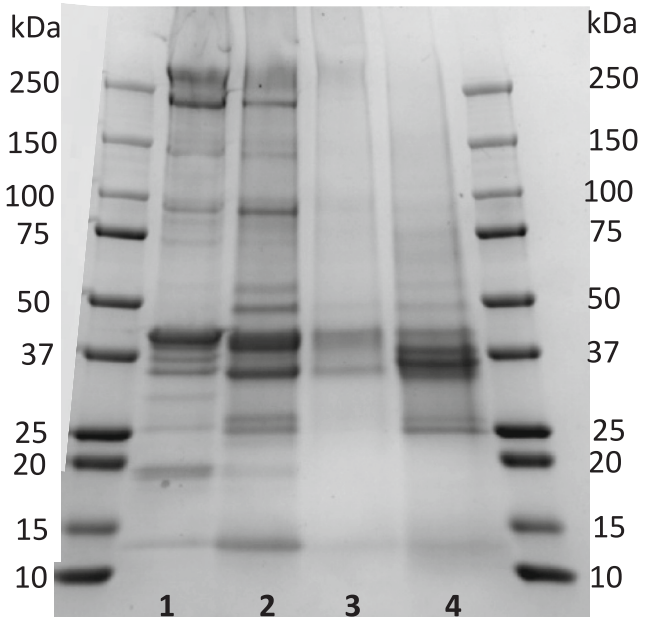

Fig. 2. Polypeptide profiling was performed on freeze-dried biomasses recovered from 3 % pre-salting brine (SB) and spice brine (SP) after initial treatment (lanes 2 and 4) and subsequent Dissolved Air Flotation (DAF). Four conditions were studied: 1) SB at its native pH (6.6); 2) SB at pH 4.7 (SB-A); 3) SP at its native pH (6.3); and 4) SP with pH adjusted using vinegar brine and HCl to pH 4.2 (SP-A). Electrophoresis was conducted using Mini-protean TGX 4–20 % precast gels from Bio-Rad Laboratories, USA. Protein bands were visualized by staining with Coomassie Brilliant Blue G-250, with each well loaded with 20 μg of protein. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

used to induce isoelectric precipitation.

The analysis of amino acid composition revealed that glutamic acid + glutamine and aspartic acid + asparagine were the predominant amino acids in all the freeze-dried biomasses except for SP-A, glutamic acid and aspartic acid are important for umami flavor. In SB-derived biomasses, lysine and leucine showed concentrations ranging from 70 to 97 mg/g while in biomasses from SP, levels were 16–51 mg/g. The significantly higher contents of these amino acids in SB and SB-A in SP and SP-A is attributed to the significantly ($p < 0.05$) higher protein content in the two former (Table 2). According to the same logics, all amino acids, except for cysteine, were significantly ($p < 0.05$) less enriched in SP compared to SP-A.

Despite the significant differences in amino acid content between biomasses originating from SB and SP, the proportion of essential amino acids (EAA) to total amino acids (TAA) remained nearly equal in all biomasses, ranging from 0.45 to 0.46. These values closely align with reported levels for beef and fish fillets; 0.47 and 0.45, respectively (Sosulski and Imafidon, 1990). Further, the EAA to TAA ratio in protein isolates recovered from cod, salmon, and herring by-products using the pH shift process has been reported as 0.47 (Abdollahi and Undeland, 2018). The present study highlights that the recovered biomasses possess a well-balanced profile of EAA suitable for human consumption; they all surpassed the requirements set by FAO/WHO for adults (WHO/FAO/UNU: Protein and amino acid requirements in human nutrition, 2007).

The total fatty acid (TFA) content in biomasses from SB and SB-A was found to be 51 and 34 mg/g, while in biomasses from spice brine, SP and SP-A, TFA were 71 and 41 mg/g. The LC monounsaturated fatty acid (MUFA) C22:1 (n-9) was the most abundant, followed by C16:0 and C18:1 (Table 3). Among fatty acid (FA) types, SFA, MUFA and PUFA constituted 28–30 %, 60.4–60.7 % and 7.3–9.4 %, respectively, in SB-A and SB. In SP-A and SP, these values were in the range of 27.7–27.9 %,

Table 2
Amino acid content (mg/g) of freeze-dried biomasses recovered with/without acidification and DAF from pre-salting (SB) and marination spice brines (SP). Data are given as average value ±SD ($n = 2$). Data within the same row having different uppercase letters are significantly different ($p < 0.05$).

mg/g	3 % salt brine (SB)	3 % salt brine acidified (SB-A)	Spice brine (SP)	Spice brine acidified using vinegar brine and HCl (SP-A)	FAO/WHO adult (mg/g protein)
Lysine	73.9 ± 1.6 ^{A}	70.9 ± 3.1 ^{A}	20.6 ± 1.0^{C}	35.5 ± 2.0^{B}	45
Arginine	47.6 ± 1.2 ^{A}	36.4 ± 0.4^{B}	5.9 ± 0.1^{D}	9.9 ± 0.2^{C}	
Histidine	18.4 ± 0.0 ^{A}	19.3 ± 0.0 ^{A}	0.0 ± 0.0^{C}	2.4 ± 0.6^{B}	15
Glycine	31.0 ± 0.1^{B}	33.8 ± 0.1 ^{A}	8.4 ± 0.3^{D}	11.5 ± 0.6^{C}	
Cysteine	3.7 ± 0.0 ^{A}	3.5 ± 0.0 ^{A}	1.6 ± 0.0^{B}	1.5 ± 0.1^{B}	
Serine	32.1 ± 0.3 ^{A}	31.0 ± 0.3 ^{A}	8.6 ± 0.2^{C}	13.1 ± 0.7^{B}	
Alanine	43.8 ± 1.0 ^{A}	46.4 ± 1.0 ^{A}	10.9 ± 0.0^{C}	19.0 ± 1.1^{B}	
Threonine	36.9 ± 0.6 ^{A}	36.1 ± 0.6 ^{A}	10.3 ± 0.1^{C}	15.8 ± 0.8^{B}	23
Glutamic acid + glutamine	97.2 ± 0.2 ^{A}	84.7 ± 2.3^{B}	26.7 ± 0.1^{D}	51.0 ± 1.6^{C}	
Aspartic acid + aspartine	84.2 ± 0.7 ^{A}	80.5 ± 1.4 ^{A}	20.8 ± 0.5^{C}	32.7 ± 1.2^{B}	
Proline	29.6 ± 0.4 ^{A}	27.2 ± 0.4^{B}	7.8 ± 0.2^{D}	10.0 ± 0.4^{C}	
Valine	42.3 ± 0.4 ^{A}	43.1 ± 0.7 ^{A}	11.0 ± 0.0^{C}	16.1 ± 0.7^{B}	39
Methionine	25.5 ± 0.9 ^{A}	22.3 ± 0.4^{B}	5.4 ± 0.1^{D}	8.6 ± 0.9^{C}	17
Tyrosine	31.7 ± 0.8 ^{A}	28.8 ± 0.4^{B}	9.4 ± 0.1^{D}	11.8 ± 0.2^{C}	
Isoleucine	34.3 ± 0.7 ^{A}	34.8 ± 0.8 ^{A}	10.5 ± 0.1^{C}	14.6 ± 0.7^{B}	30
Leucine	67.9 ± 1.8 ^{A}	61.1 ± 0.2^{B}	16.5 ± 0.3^{D}	27.8 ± 1.0^{C}	59
Phenylalanine	31.5 ± 0.4 ^{A}	33.6 ± 0.0^{B}	8.5 ± 0.2^{D}	11.0 ± 0.6^{C}	19
TAA	731.5	693.6	182.8	292.3	
EAA	330.6	321.2	82.8	131.8	
EAA/TAA	0.45	0.46	0.45	0.45	

TAA, total amino acids; EAA, essential amino acids

Table 3
Fatty acid profile (mg/g) of biomasses recovered with DAF +/− acidification of 3 % pre-salting brine (SB) and spice brine (SP). Data are shown as mean ± SD ($n = 2$). Data within the same row having different uppercase letters are significantly different ($p < 0.05$).

	Fatty acid content (mg/g powder)			
	3 % pre-salting brine, native pH (SB)	3 % pre-salting brine, pH 4.7 (SB-A)	Spice brine (SP)	Spice brine acidified using vinegar brine and HCl (SP-A)
C12:0	0.1 ± 0.0^{B}	0.0 ± 0.0^{B}	0.1 ± 0.0 ^{A}	0.0 ± 0.0^{B}
C14:0	6.1 ± 0.5 ^{A}	4.4 ± 0.1^{B}	6.0 ± 0.0 ^{A}	3.7 ± 0.5^{B}
C16:0	10.4 ± 0.7^{B}	7.7 ± 0.3^{C}	14.0 ± 0.0 ^{A}	8.2 ± 1.0^{BC}
C18:0	0.6 ± 0.0^{BC}	0.4 ± 0.0^{C}	1.7 ± 0.0 ^{A}	0.8 ± 0.1^{B}
C16:1 (n-7)	2.9 ± 0.0^{B}	2.0 ± 0.0^{C}	4.5 ± 0.0 ^{A}	2.6 ± 0.3^{BC}
C17:1 (n-7)	0.1 ± 0.0^{B}	0.1 ± 0.0^{B}	0.3 ± 0.0 ^{A}	0.1 ± 0.0^{B}
C18:1 (n-7)	0.7 ± 0.1^{B}	0.6 ± 0.0^{B}	1.6 ± 0.0 ^{A}	0.8 ± 0.1^{B}
C18:1 (n-9, n-12)	5.8 ± 0.3^{B}	3.9 ± 0.0^{B}	11.9 ± 0.0 ^{A}	6.2 ± 0.8^{B}
C20:1 (n-9)	9.0 ± 0.4 ^{A}	5.9 ± 0.0 ^{B}	6.6 ± 0.0 ^{B}	4.6 ± 0.6 ^{B}
C20:1 (n-15, n-13)	0.8 ± 0.0 ^{A}	0.5 ± 0.0^{C}	0.7 ± 0.0^{B}	0.4 ± 0.1^{C}
C22:1 (n-9)	17.3 ± 1.1 ^{A}	11.4 ± 0.0^{BC}	13.6 ± 0.3^{B}	9.1 ± 1.2^{C}
C18:2 (n-6)	0.8 ± 0.0^{B}	0.5 ± 0.0^{C}	2.4 ± 0.0 ^{A}	1.0 ± 0.1^{B}
C18:3 (n-3)	0.5 ± 0.0^{B}	0.3 ± 0.0^{C}	1.0 ± 0.0 ^{A}	0.5 ± 0.1^{B}
C20:5 (n-3)	2.0 ± 0.1^{C}	1.1 ± 0.0^{D}	5.2 ± 0.0 ^{A}	3.0 ± 0.4^{B}
C22:6 (n-3)	3.3 ± 0.3^{B}	1.6 ± 0.0^{C}	8.5 ± 0.0 ^{A}	4.6 ± 0.6^{B}
% SFA	28.4	30.9	27.9	27.7
% MUFA	60.7	60.4	50.2	52.4
% PUFA	9.4	7.3	18.8	17.6
% n-3 PUFA	9.4	7.3	18.8	17.6
% n-6 PUFA	1.6	1.2	3.0	2.1
n-3:n-6	6.2	5.2	5.7	7.8

50–52 % and 17.6–18.8 %, respectively. Corresponding values for MUFA and PUFA in herring tissue are reported as 44–46 % and 20–32 %, respectively (Jensen et al., 2007). LC-MUFA account for 44.1–44.8 % and 26–31 % of MUFA in the SB/SB-A and SP/SP-A, respectively. LC-MUFA such as C20:1 n-9 and C22:1 recorded in this study are associated with the improvement of several inflammatory cytokines and the gut microbiota environment by stimulating short-chain fatty acids' production (Tsutsumi et al., 2021) as well as the glucose/lipid homeostasis (Yang et al., 2011). The ratio between n-3 PUFA and n-6 PUFA in all biomasses (5.2–7.8) was lower than that reported for herring tissue (9.0–11.5) (Jensen et al., 2007). Also, the relative level of LC n-3 PUFAs based on total FA in the biomasses was much lower (6.6–16.6 %) than reported e.g. for herring fillets (44–49 %) (Wu et al., 2022), which could be due to the accumulation of LC n-3 PUFA particularly in the membranes rather than the neutral lipid deposits (Sissener et al., 2016); the latter which are more easily released into process waters. Overall, the recovered biomasses however contain a good balance of beneficial fatty acids of both MUFA and PUFA families.

3.1.1. Volatile compounds in freeze-dried biomasses

Using external standards, we quantified 14 volatiles, including butanal, 2-butanone, 2-methyl-2-butanal, 3-methyl-2-butanal, 1-penten-3-one, 3-methyl-1-butanol, 2-methyl-1-butanol, hexanal, t-2hexenal, heptanal, 2,5-dimethylpyrazine, c-4 heptenal, 2,4 heptadienal, and 2,4 nonadienal in the freeze-dried biomasses SB and SB-A (Table 4). Among these, 2-methyl-2-butanal, 3-methyl-2-butanal, t-2-hexenal, heptanal, 2,5-dimethylpyrazine, and c-4 heptenal were found at levels below 100 ng/g in SB and SB-A. The remaining volatiles were found at levels ≤211 ng/g, except for butanal, 2-butanone, and 1-penten-3-one, which ranged from 300 to 770 ng/g.

The amount of each volatile differed significantly ($p < 0.05$) between biomasses from SB recovered at the native pH (6.6) and pH 4.7. Butanal, hexanal, t-2-hexenal, and c-4 heptenal contents were higher in SB-A than SB, while for the other volatiles, the order was opposite. More investigations are needed to explain these differences.

All mentioned volatiles, except for heptanal and c-4 heptenal, were also detected in biomasses derived from SP, and the amounts were significantly ($p < 0.05$) higher in the biomass recovered using HCl and vinegar brine (SP-A) compared to that recovered at native pH. The content of hexanal, 2- butenal, and 3-methyl-2-butanal was found to be 325, 557 and 829 ng/g, respectively in SP-A.

Further to recovery pH, the amount of volatiles released from each biomass type could be dependent on its lipid content. The contents of lipids ranked the four biomasses according to SP > SB > SP-A > SB-A. Higher lipid levels could retain more hydrophobic volatiles such as

Table 4
Volatiles quantified using GC–MS (ng/g powder) of freeze-dried biomasses recovered from 3 % pre-salting brine (SB, SB-A) and spice brine (SP, SP-A). Data are shown as mean ± SD ($n = 3$).

Biomass	butanal	2-butanone	2-methyl-2-butanal	3-methyl-2-butanal	1-penten-3-one	3-methyl-1-butanol	2-methyl-1-butanol-	hexanal	2-hexanal	heptanal	2,5 dimethyl-pyrazine	c-4 hepta-nal	2,4 heptadi-enal	2,6 nonadi-enal
3 % pre-salting brine (SB)	141.3 ± 12.4^{C}	770.3 ± 82.6^{A}	27.8 ± 2.4^{B}	<LOD	562.4 ± 67.2^{A}	201.5 ± 22.2^{A}	190.1 ± 11.5^{A}	114.1 ± 16.8^{B}	6.8 ± 0.6^{B}	<LOD	24.6 ± 1.5^{A}	<LOD	211.2 ± 15.9^{A}	115.4 ± 7.8^{A}
3 % pre-salting, pH 4.7 (SB-A)	307.9 ± 56.0^{B}	274.9 ± 61.6^{B}	14.0 ± 2.6^{B}	11.9 ± 5.9^{C}	138.5 ± 6.8^{B}	61.5 ± 16.4^{B}	18.7 ± 4.3^{B}	153.7 ± 60.4^{B}	27.8 ± 10.5^{A}	20.0 ± 3.4^{A}	6.4 ± 0.3^{C}	28.4 ± 15.2^{A}	185.0 ± 13.9^{AB}	94.0 ± 5.9^{A}
Spice brine (SP)	71.3 ± 3.6^{C}	78.0 ± 2.1^{C}	17.7 ± 0.7^{B}	242.2 ± 8.7^{B}	8.5 ± 1.1^{C}	5.2 ± 0.2^{B}	5.7 ± 0.1^{C}	9.1 ± 1.1^{C}	<LOD	<LOD	7.0 ± 0.3^{C}	<LOD	45.4 ± 1.0^{C}	29.2 ± 0.9^{B}
Acidified Spice brine using vinegar brine and HCl (SP-A)	557.7 ± 34.2^{A}	53.1 ± 9.3^{C}	95.2 ± 4.3^{A}	829.6 ± 33.9^{A}	104.3 ± 10.6^{B}	17.0 ± 0.4^{B}	18.6 ± 0.3^{B}	325.8 ± 18.4^{A}	<LOD	10.6 ± 4.0^{B}	20.3 ± 0.1^{B}	<LOD	157.3 ± 4.2^{B}	99.7 ± 2.6^{A}

Means with different superscript letters within the same column for each water type indicate significant differences among these samples ($p < 0.05$).

heptanal, i.e., preventing their release into the headspace. For example, using a homologous series of hydrocarbons, aldehydes, ketones, and alcohols, Jo and Ahn (Jo and Ahn, 1999) found a linear decrease in flavor release with increasing fat content of an emulsion.

The straight-chain aldehyde hexanal is characterized by herbaceous and green-grassy aromas and is derived from the lipid oxidation of n-6 PUFA (Collomb and Spahni, 1996); it is widely recognized as a lipid oxidation index (Shahidi, 2012). Butanal can form from xx fatty acid during lipid oxidation (Grebenteuch et al., 2021) and has earlier been detected in the headspace when heating herring at 200 °C (Yasuhara and Shibamoto, 1995). Also 1-penten-3-one is a well-known lipid oxidation product which has been found to correlate well with peroxide and anisidine values in herring oil and it stems from n-3 PUFA (Aidos et al., 2002b). It has been reported to be associated with pungent, glue-like, rancid, green and fishy odor in both fish oil and fish muscle (Hammer and Schieberle, 2013; Lee et al., 2003). Another aldehyde quantified in high levels in the SP-A was 3-methyl-2-butanal which is formed from leucine via Strecker degradation (Smit et al., 2009). Its odor has been described as chocolate-like (Lin et al., 2014). Ketones, such as 2-butanone, can originate from lipid oxidation reactions; either chemically-induced autoxidation and/or enzymatic β-oxidation (Wong, 2018). 2-butanone is linked to fruity flavours (Lin et al., 2014). It has earlier been described that the storage of raw muscle products may also lead to the generation of oxidation-derived aldehydes, methyl alcohols, and ketones due to the catabolism of branched-chain amino acids and pyruvate (Estévez et al., 2003).Altogether, the volatile compound profile indicates development of lipid oxidation either during generation of the brines or their valorization with DAF, with and without acidification. Numerous reasons could explain this observation, such as the combination of heme-proteins, lipoxygenase, salt and highly unsaturated fatty acids (Mariutti and Bragagnolo, 2017; Richards and Hultin, 2002; Wu et al., 2022). The exposure to air during the DAF treatment is another critical factor, which could be mitigated by using e.g., nitrogen gas in this recovery process.

3.2. Emulsion and foaming properties of freeze-dried biomasses

The emulsification capacity of proteins in a mixture is crucial for their potential application in emulsion-based food products. In this study, we determined the emulsion activity index (EAI) and emulsion stability index (ESI) of freeze-dried biomasses over a pH range of 3–11 (Fig. 2A-B). Biomasses from spice brine (SP, SP-A) exhibited significantly higher EAI values compared to those from 3 % pre-salting brine (SB, SB-A). Notably, all protein powders showed a minimum EAI at pH 5, indicating an isoelectric region. At this minimum, SP and SP-A showed EAI of 31 and 15 m^2/g, respectively, whereas SB and SB-A remained identical at 3 m^2/g. Below and above this pH, EAI levels generally increased for all biomasses.

For SB and SB-A, the EAI was approximately 20 m^2/g for both biomasses at pH 3 while it was 25 and 22 m^2/g, respectively, at pH 11. It is noteworthy that these EAI-values were considerably lower than those obtained for freeze-dried protein isolate from herring solid side streams recovered using the pH shift method (38–66 m^2/g over pH 3–11) (Abdollahi and Undeland, 2018). Despite the relative similarity in polypeptide profiles between the SB-derived biomasses and herring side stream protein isolate, the lower EAI values for the former could be attributed to their more complex proximate composition, e.g. with significant levels of salt and polyphenols. Also, a somewhat higher level of globular than fibrillar proteins is evident from a comparison of SDS-PAGE results across studies.

SP exhibited an EAI of 85 m^2/g at pH 11, while at pH 3, it showed an EAI of 63 m^2/g. In contrast, SP-A had a slightly higher EAI at pH 3 (80 m^2/g) compared to at pH 11 (75 m^2/g). As for SB and SB-A, there were lower EAI values for both biomasses at pH 5 compared to at extreme acid and alkaline pH's, reflecting that hydration/solubility of proteins plays a crucial role for their capacity to migrate to an oil/water interface, forming a film or layer, which is necessary to stabilize an emulsion (Shevkani et al., 2015). At pH values far away from the pI, proteins carry a net positive or negative charge, respectively, enhancing their solubility and ability to migrate to the oil-water interface. This explains the higher EAI values observed at pH 3 and 11. Beyond solubility, several factors contribute to the emulsification capacity of proteins, including size, shape, conformation, steric factors, polarity of amino acid side chains, as well as the presence of salts, lipids and carbohydrates. The higher EAI values observed in the spice brine-derived biomass SP and SP-A compared to SB and SB-A could be attributed to its higher salt concentration (Zhang et al., 2022) and the higher amount of peptides (Wang et al., 2022); the latter being a result of extensive proteolysis during the prolonged marination period. That higher salt levels could enhance emulsification properties was recently also reported for soy

β-conglycinin (Zhang et al., 2022). Regarding peptides, these have a higher mobility and flexibility compared to larger sized proteins, why they more readily can adsorb at interfaces. Generally, the reduced tertiary and quaternary structures of low molecular weight peptides leads to greater surface activity, which is crucial for functional properties like emulsification.

ESI values of SP and SP-A ranged from 3.4 to 33.1 min and revealed a similar pattern as for EAI, with a minimum in the isoelectric region. For biomasses from pre-salting brines (i.e., SB and SB-A), there was surprisingly a peak in ESI at pH 5, and overall, values ranged from 11 to 38 min, with lowest values seen at pH 3 and 11. It has earlier been reported that the emulsifying capacity of casein hydrolysates increased at the isoelectric point, while with whey protein hydrolysates the emulsifying capacity increased at alkaline pH (Chobert et al., 1988). Thus, the different behaviors could be due to protein compositional differences.

Foaming capacity (FC) and foaming stability (FS) are essential parameters that provide insights into the potential application of proteins in food products which need to accommodate air. Overall, SP and SP-A demonstrated significantly higher FC and FS than SB and SB-A. This was likely due to the same reason as discussed above; i.e., a larger amount of soluble peptides and higher hydration compared to the others. Further, SP and SP-A showed a higher FC (25 %) and FS (23 %) at pH 11, compared to pH 3 (20 % and 17 %, respectively) while for SB, FC values obtained at pH 11 and 5 were significantly different from those at pH 7 and 3 (Fig. 3C-D). The higher amount of salt in SP and SP-A could affect foaming properties in different ways, examples being through reduced surface tension and reduced protein-protein repulsions, the latter enhancing the formation of a protein network at the air-water interphase (Zhang et al., 2024). Aligned with the present observations, Kandasamy, Karuppiah (Kandasamy et al., 2012) also reported salt-dependent

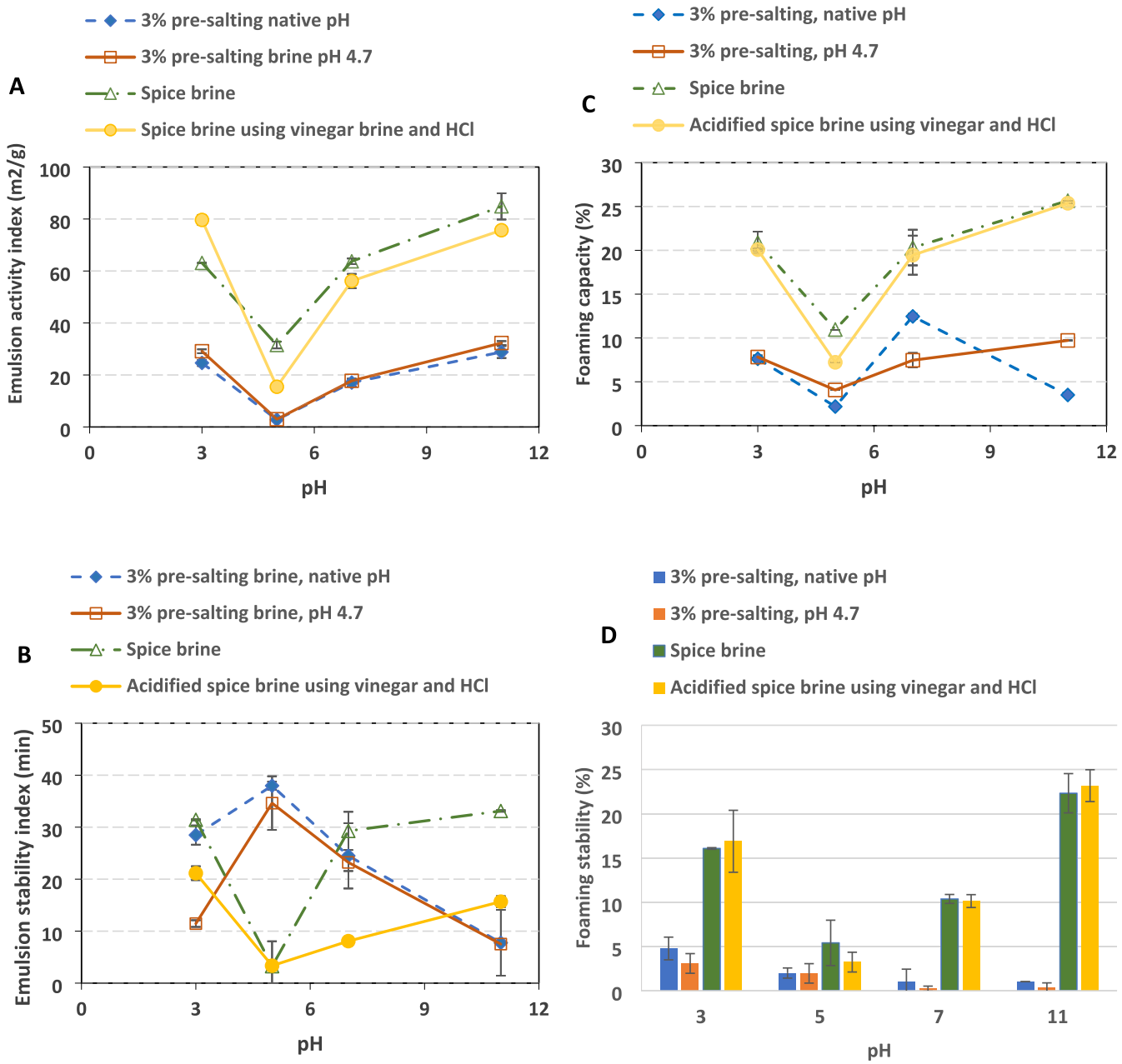

Fig. 3. Emulsion activity index (EAI), emulsion stability index (ESI), foaming capacity (FC) and foaming stability (FS) of biomasses recovered using DAF from 3 % pre-salting brine at native pH (SB) and pH 4.7 (SB-A) as well as biomasses derived from spice brine at native pH (SP) and pH 4.2 (SP-A). Data show mean values (±SD) ($n = 2$).

enhancement of foaming capacity of protein concentrates extracted from three green seaweed species of *Enteromorpha*.

3.2.1. Fourier transform infrared (FT-IR) analysis of freeze-dried biomasses

The FT-IR spectra results, depicted in Fig. 4, reveal distinctive absorption bands for all dried biomasses. Notably, characteristic peaks are observed at 3277–3290 cm-1 (Amide A, N—H, or O—H stretching), 1627–1645 cm-1 (Amide *I*, *C* = O and C = N stretching), 1515–1532 cm-1 (Amide II, C—N stretching and N—H bending), and 1231–1235 cm-1 (Amide III). The Amide I absorption zone, in particular, is crucial for evaluating the secondary structure of proteins, encompassing presence of α-helix, β-sheet, β-turn, and random coil. In SB and SB-A, the amide I peak was more intense but shifted towards lower wavelength (1629 cm-1) for SP and SP-A (1641 cm-1). The former indicates higher presence of α-helix structure of myosin as also shown in the polypeptide profiling, while the latter point to the extensive hydrolysis that had occurred in SP and SP-A (Carbonaro and Nucara, 2010). Similarly, Amid II and III peaks were shown for all biomasses at 1515 cm-1 and 1212 cm-1, respectively, with higher intensities for SB/SB-A than SP/SP-A. This confirm the extensive proteolysis in the latter, but likely also reflects structural changes occurring in the presence of high salt concentrations as used during the marination in spice brines (Usoltsev et al., 2020).

4. Conclusion

This study conducted a comprehensive analysis of biomasses recovered from primary and secondary herring brines using DAF, without and with isoelectric protein precipitation. The focus was on crude composition, profiles of polypeptides, amino acids, fatty acids and volatiles, as well as functional properties. Several key findings emerged from the investigation. For example, biomasses from herring pre-salting brines (SB/SB-A) were higher in protein and lower in ash than those from secondary spice brines (SP/SP-A). The latter were enriched in lower molecular weight peptides/polypeptides compared to pre-salting brines, reflecting severe proteolysis during herring maturation. Glutamic acid and aspartic acid were among the predominant amino acids, which are important for umami flavor. Essential amino acids of biomasses made up 45–46 % of total amino acids and among fatty acids, there were high levels of particularly LC MUFA, but also up to 15 % of LC n-3 PUFA. Detected volatile compounds reflected that both lipid oxidation and Strecker degradation had taken place e.g., during brine fractionation and/or biomass drying. Biomasses from spice brine generally exhibited higher emulsion activity and foaming capacity compared to those from 3 % pre-salting brine, likely due to higher levels of peptides. The latter was also confirmed via FTIR spectra, revealing less secondary structures in spice brine-derived biomasses.

The present findings suggest that currently wasted aqueous seafood side streams can be converted into new types of protein ingredients, which in different ways can be beneficial to the seafood industry and beyond. These ingredients can have diverse applications such as emulsifiers, binders, and protein fortifiers. They can also be directed towards bio-based films, or enzymatic hydrolysis to produce flavor enhancers and bioactive peptides. Through upcycling of aqueous side streams, costs for wastewater handling may be reduced or even omitted, and second, incorporating the new side stream-derived marine biomasses into product formulations can enhance their nutritional value, seafood flavor, emulsifying as well as foaming properties. The study's insights can guide further research and innovation and contribute to the development of a more sustainable seafood industry.

CRediT authorship contribution statement

Bita Forghani: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Tore C. Svendsen:** Writing – review & editing, Resources, Methodology, Formal analysis. **Anubhav Pratap-Singh:** Writing – review & editing, Resources, Formal analysis.

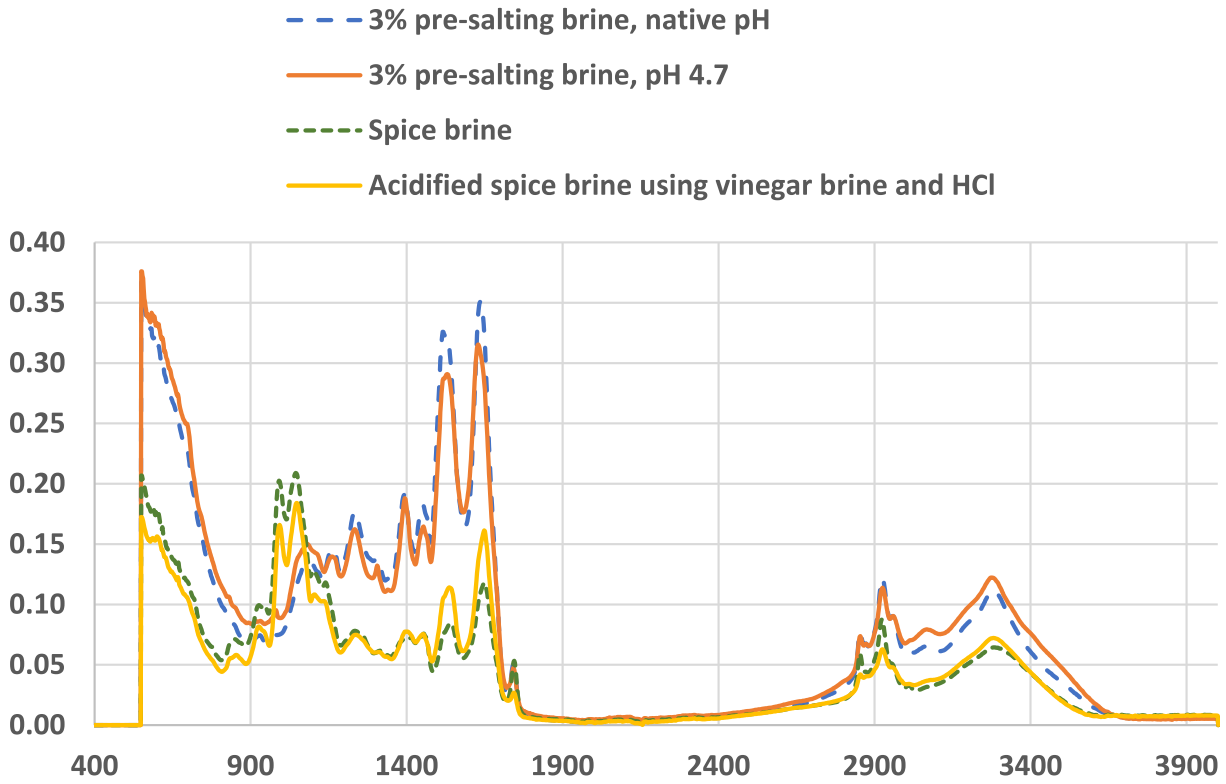

Fig. 4. FTIR spectrum of biomasses recovered using F-DAF from 3 % pre-salting brine at native pH (SB) and pH 4.7 (SB-A) as well as biomasses derived from spice brine at native pH (SP) and pH 4.2 (SP-A).

Charlotte Jacobsen: Writing – review & editing, Resources, Formal analysis. **Ingrid Undeland:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization.

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.

Acknowledgement

This study was funded by Marie-Curie and VINNOVA (VINNMER) (project #2016-04142, awarded to Bita Forghani), the Swedish Board of Agriculture/European Maritime and Fisheries fund (EMFF) (Aquastream project, #2016-844-3, granted to Ingrid Undeland) and by the Bio Based Industries Joint Undertaking (JU) under grant agreement No 837726. The JU receives support from the European Union's Horizon 2020 Research and Innovation Programme and the Bio Based Industries Consortium. This output reflects only the authors' views, and the JU cannot be held responsible for any use that may be made of the information it contains.

Data availability

Data will be made available on request.

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