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Chemical Characterization, Antioxidant and Enzymatic Activity of Brines from Scandinavian Marinated Herring Products

Nina Gringer1,*, Ali Osman2, Henrik H Nielsen1, Ingrid Undeland1 and Caroline P Baron1

1Technical University of Denmark, Division of Industrial Food Research, National Food Institute, DK-2800 Kgs. Lyngby, Denmark
2Chalmers University of Technology, Department of Chemical and Biological Engineering, Food Science, SE 412 96 Gothenburg, Sweden

Abstract

Brines generated during the last marination step in the production of marinated herring (Clupea harengus) were chemically characterized and analyzed for antioxidant and enzyme activities. The end-products were vinegar cured, spice cured and traditional barrel-salted herring with either salt or spices. The chemical characterization encompassed pH, dry matter, ash, salt, fatty acids, protein, polypeptide pattern, iron and nitrogen. The antioxidant activity was tested with three assays measuring: iron chelation, reducing power and radical scavenging activity. The enzymatic activity for peroxidase and protease were also tested. Results revealed that the brine can contain up to 56.7 mg protein/mL, up to 20.1 mg fatty acid/mL, good antioxidant activity, high amounts of the antioxidative amino acids lysine, alanine, and glycine, and high enzymatic activity. The potential of using the protein-rich fraction with biological activity from brines from the marinated herring production was demonstrated in this work.

Keywords: Herring (Clupea harengus); Brine; Wastewater; Antioxidant (iron chelating, reducing power and ABTS radical scavenging); Enzymatic (peroxidase and protease)

Introduction

The food sector produces large volumes of waste, both solids and liquids, resulting from the production, preparation, and consumption of food and this represent an important loss of valuable biomass and nutrients. In addition, with the growing population and the concerns about environmental pollution, today’s society sets focus on our limited resources and on optimizing the utilization of the available raw materials. Consequently, there is a considerable emphasis on the recovery, recycling and upgrading of our organic waste in order to extract as much value as possible from them [1-3]. Therefore, it is important to also propose new solutions to food producers to optimize existing processes in order to both reduce the amount of waste generated and extract more value from the residual raw material that is wasted.

In the fish industry, up to 50% of the raw material is commonly discarded [4], and there is an increasing interest within the seafood sector to explore new possibilities for their utilization [5,6]. In Norway, almost 800 000 tons of by-products were generated in 2009, of which approximately 77% were exploited and the remaining 23% were dumped. More than 80% of the exploited by-products were used for the production of low value-added products such as fish meal, silage and feed whereas only about 15% were used for human consumption [7]. Research has shown that marine by-products contain compounds such as minerals, fatty acids, amino acids, polysaccharides and proteins with interesting biological activity [8-11]. Sathivel et al. [12] demonstrated that protein hydrolysates made from herring by-products (head and gonad) may serve as a good source of desirable quality peptides and amino acids.

Besides the solid by-products, a large amount of liquid waste is produced in the fish industry. A well-known example is the wash water from surimi production. Stine et al. [13] showed that proteins in such wastewater could be recovered and added back to the surimi without affecting the end-product quality. Another type of product which, during production, generates large amount of liquid waste is the marinated herring, a traditional Scandinavian product. The liquid waste generated from the production of these marinated products can in total reach a volume of more than 700 L per 100 kg herring produced; of which ~200 L come from the maturation step and onwards. Since the European Union annually lands almost 960 000 tons of herrings, of which a large proportion ends up as marinated product [14], there is a huge amount of liquid by-products available for value adding from this production in Europe. A crucial part of this type of production is the maturation step in which fillets or whole fish can be stored in salt brine from a few months and up to two years. During this period, many biochemical reactions take place liberating amino acids, peptides, enzymes and lipids into the brine [10]. The brine from marinated herring has been characterized in order to better understand the ripening process of barrel-salted herring [8,9,15,16]. Christensen et al. [17] tried to re-use such brine in a fresh batch of traditional barrel-salted herring in order to speed up the maturation time, but this study showed no effect of the added brine on the processing time. Apart from these studies, a thorough characterization of brines containing spices and desalting brines has, to the best of our knowledge, never been performed. Furthermore, investigation of the potential utilization of herring marinating brines has not been previously reported.

In this work, we aimed to characterize brines from the last steps in the production of marinated herring (i.e. from the maturation step and onwards) in order to identify compounds with any potential commercial value that can be exploited from this by-product streams. In addition to the basic chemical characterization, antioxidant activity and enzymatic activity, i.e. peroxidase and protease, were also investigated. Four types of marinated end-products were considered; vinegar cured, spice cured and two traditional barrel-salted products; one with salt and one with spices.

*Corresponding author: Nina Gringer, Technical University of Denmark, Division of Industrial Food Research, National Food Institute, DK-2800 Kgs. Lyngby, Denmark, Tel: +45 45 25 66 24; Fax: +45 45 88 47 74; E-mail: ngri@food.dtu.dk

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Materials and Methods

All chemicals and reagents were of analytical grade and were purchased from Merck (Darmstadt, Germany) or Sigma-Aldrich (Steinheim, Germany). See-Blue pre-stained standard was purchased from Life Technologies (Nærum, Denmark) and Coomassie brilliant blue from AppliChem (Darmstadt, Germany).

Herring brine production

Brines from the marination steps in the production of marinated herring (*Clupea harengus*) were supplied from Lykkeberg A/S (Hørve, Denmark), and were all prepared according to Lykkeberg A/S production protocols from herring caught in the North Atlantic. Six brines were obtained from the production of four different types of marinated products, as shown in Table 1. Four brines were original brines; i.e. the brines from vinegar cured fillets (VC), spice-cured fillets (SC), traditional salted whole herring (TSa) and traditional salted spice-cured whole herring (TSp). The two remaining brines were desalting brines from TSa and TSp (D-TSa, D-TSp). TSa and TSp results from salting whole herring, filleting and then placing the fillets back in the brine for additional ripening, whereas SC and VC are produced as fillets or bites. Brine samples were divided into appropriate aliquots, transported on ice to the laboratory, stored at -80°C and thawed on ice water prior to experiments.

Proximate composition

The pH was measured directly in the brine using a Metrohm 827 pH meter (Switzerland). Dry matter (DM) content of the different brines was measured by a two-step evaporation of water at 60°C for 24 h followed by 105°C for 24 h. The %-DM was calculated as (mass of dry sample * 100) / (mass of wet sample). Ash content was measured by burning at 600°C for 24 h, and is reported as (mass of ash * 100) / (mass of wet sample). The salt content was measured by potentiometric titration of chloride ions using AgNO₃, according to the AOAC standard method [18]. The iron content was measured by complete digestion of the sample and subsequent analysis by inductively coupled plasma mass spectrometry, ICP-MS (Perkin-Elmer SCIEX, ELAN 6000). For sample digestion, 1 g of brine was mixed with 5 ml HNO₃ (67-69%), 3 ml H₂O₂ (30%) and 0.5 ml HCl (37%), and digested for 10 min in a microwave (Multiwave 3000, Anton Paar, Austria) at 1400 W, according to the Application Notes from Anton Paar. Prior to ICP-MS analysis, H₂O was added to a sample volume of 10 ml. The iron content was calculated from a standard curve made with Titrisol® iron standard (Merck, Darmstadt, Germany).

The fatty acid analysis of the different brines was completed by solvent extraction followed by methylation and detection by gas chromatography mass spectrometry, GC-MS, of the different fatty acids [19,20]. In short, 20 mL ice-cold chloroform: methanol (1:2, containing 0.04% butylated hydroxytoluene), 40 µL standard (C17, 2 mg/mL, dissolved in toluene) and 2 mL of raw brine sample was vortexed for 20 sec. Subsequently 8 ml of NaCl (0.05%) was added and vortexed for 30 sec. and centrifuged (Heraeus Multifuge 1 S-R, Thermo scientific, Sweden) for 6 min at 2 000g, 4°C. The chloroform phase was collected and left to evaporate under nitrogen gas and re-dissolved in 2 mL toluene. Then, 2 mL of 10% (v/v) acetyl chloride in methanol were added and vortexed for 20 sec. The tubes were placed in a water bath (70°C) for 2 h and then cooled under cold running water. One mL of dH₂O and 2 mL of petroleum ether were added and vortexed for 20 sec and then centrifuged at 1000 g for 5 min. The upper organic phase, i.e. petroleum ether, was collected and evaporated under nitrogen at 40°C. Evaporated samples were then dissolved in 1 mL isooctane, from which 200 µL were used for GC-MS analysis.

Methylated fatty acids were analyzed by GC-MS using Agilent Technologies 7890A GC system connected to Agilent Technologies 5975C inert XL EI/CI MSD with triple axis detector (Kista, Sweden). One µL of the methylated sample was injected into a split/spillless capillary injector (275°C), with a split ratio of 15:1. Fatty acid separation was conducted on a DB-WAX column from Agilent technologies (30 m×250 µm×0.25 µm) using helium as carrier gas at a flow rate of 1 mL/min. The oven was initially programmed at 100°C, constant increase of 4°C/min to 250°C, kept constant at 250°C for 4 minutes; total run time was 41.5 min. During the analysis, the MS temperature was 230°C, the MS quadrupole was 150°C and the electron energy was 70 eV; all data were acquired in scan mode with an m/z range of 35 – 500. The GC chromatograms and the MS spectra were analyzed by MSD chemstation G1791EA software provided by Agilent Technologies. The different fatty acids were identified by comparing with fatty acid standards. The standard C17 was used to estimate the amount of each fatty acid (mg/ml) in the samples. Total fatty acid content was calculated as the sum of all detected fatty acids.

The nitrogen content was determined by Kjeldahl method, according to the AOAC standard method [21]. The protein content (mg/mL) of the brines was obtained using the BCA kit (Thermo Scientific, Pierce®, Rockford, USA) with Bovine Serum Albumin (BSA) as standard. This protein determination assay was tested for its salt stability using a 16% salt solution (highest level found in the brines) and was not found to be affected by salt. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was conducted to separate the different protein/polypeptides according to their molecular weight. The brines were diluted to a protein concentration of 1 mg/mL and mixed with Laemmli sample buffer (1:1) with 10% DDT (v/v), boiled for 3 min and centrifuged for 3 min at 13 684 g (Heraeus™ Biofuge® Pico, Kendro, UK). Fifteen µL of the sample (7.5 µg protein) were protein was loaded onto the wells of 10% NuPAGE® Bis-Tris Gels (Life Technologies™, Denmark) and run with MOPS 3-(N-morpholino)propanesulfonic acid running buffer at 200 V for 50 min. See-Blue was used as standard (10 µL was loaded on the gel). Gels were stained with Coomassie Brilliant blue G-250 overnight and washed with a de-staining solution (15% ethanol, 24% methanol, 10% acetic acid and 0.1% acetic acid). The gels were scanned, and the protein content was estimated by comparing with the pre-stained standard (SDS-PAGE, see-blue pre-stained standard was purchased from Life Technologies (Nærum, Denmark) and Coomassie brilliant blue from AppliChem (Darmstadt, Germany).

Table 1: General product information of the six brines analyzed. The processing time for the specific samples taken for this study were ~200 days for VC and SC, and ~450 days for TSa and TSp, respectively.

| Product | Brine | Salt | Sugar | Acetic acid | Spice-mix | Processing time (days) |
|---------|-------|------|-------|-------------|-----------|-----------------------|
| Vinegar cured (fillet) | VC | yes | no | yes | No | min 35 |
| Spice-cured (fillet) | SC | yes | yes | no | Yes | min 42 |
| Traditional salted (fish†) | TSa | yes | no | no | No | 180-550 |
| Traditional salted spice-cured (fish†) | TSp | yes | yes | no | yes | 180-550 |
| Desalting brine from TSa (fillet) | D-TSa | only fresh water | | | | 1 |
| Desalting brine from TSp (fillet) | D-TSp | | | | | 1 |

† whole fish for 6 month, then fillet and placed back in the brine
5% acetic acid) until protein bands became clearly visible in a colorless gel matrix.

**Free and total amino acids**

Free amino acids and total amino acids were analyzed, according to the method described by Farvin et al. [22]. For free amino acids determination, the amino acids were derivatized using the EZ: Fast kit from Phenomenex A/S (Allerød, Denmark). Two µL of the sample were injected into the HPLC fitted with the reversed phase column EZ: Fast AAA-MS (250 x 3.0 mm, Phenomenex A/S, Allerød, Denmark), and eluted at 35°C with a flow rate of 0.5 mL/min. The mobile phase A (water) and B (methanol) both contained 10 mM ammonium formate. The gradient consisted of linear increase from 60 to 83% B in 20 min, and then the column was re-equilibrated to 60% B until the end of the run (26 min). The eluate was transferred to the on-line MS (Alligent 1100, Aligent Technology, Waldbronn, Germany) where amino acids were ionized using APCI with scanning from 100 to 600 m/z. The amino acids were quantified based on peak areas of internal standards.

For total amino acids, a sample of 500 µL brine was hydrolyzed for 1 h in a microwave (same as for iron), at 110°C at 500 W, in 500 µL of 12 M HCl in sealed ampules. The samples were diluted in NaCO3 and filtered through a 0.2 µm membrane filter before the derivatization and the analysis of amino acids content by LC-MS, as described for the free amino acids. It should be notified that this procedure did not allow the detection of methionine, tryptophan and cysteine. For both experiments, amino acid content is expressed in g/L brine.

**Antioxidant assays**

All the brines were analyzed using three assays for antioxidant activities; metal chelation, reducing power and ABTS-radical scavenging. To test the concentration dependency of the antioxidant activities, all the brines were analyzed in the crude, undiluted version and following sequential threefold dilutions, until a steady level was observed. They were measured on a spectrophotometer (Synergy 2 Multi-Mode Microplate Reader, BioTek® Instruments, Inc., Vermont, USA), and tested with high salt solution (16%) prior to analysis and none of them were affected by this salt concentration.

Iron (II) chelating activity of the different brines was assayed by the method described by Farvin et al. [23] adjusted to microplate detection. Brines subjected to the different dilutions were centrifuged (13 684 g, 3 min, Biofuge® Pico, Kendro, UK) and 100 µL of the supernatant was mixed with 135 µL dH2O in the microplate. The reaction was initiated by 5 µL of 2 mM ferrous chloride, and inhibited by 10 µL of 5 mM potassium persulfate, both in dH2O) was left in the dark over night (1100, Aligent Technology, Waldbronn, Germany) using dH2O as an equilibration medium, prior to the assay. Results were expressed as ΔAbs/min/ml.

The reducing power was measured according to the protocol described by Re et al. [24,25] with some modifications. In short, an ABTS reagent (1:1 v/v consisting of 10 mM ABTS and 5 mM potassium persulfate, both in dH2O) was used as a positive control and was subjected to the same dilutions as the samples. Results are expressed as OD734 for the different samples at the different dilutions.

The radical scavenging activity was determined according to the method described by Re et al. [24,25] with some modifications. In short, an ABTS reagent with borax buffer (0.1 M, pH 9). Fifty µL of sample were mixed with 200 µL ABTS working solution in the microplate and read after 1 minute at room temperature at 734 nm. Trolox (2.5 mM, in 96% ethanol) was used as a positive control and was subjected to the same dilutions as the samples. Controls were included consisting of dH2O instead of the reagents and pure dH2O and 96% ethanol. Results are expressed as OD591 for the different samples at the different dilutions.

**Enzymatic activity**

The brines were analyzed for two types of enzymatic activity; peroxidase and protease activity. Both methods were tested for stability with high salt solution (16%). The peroxidase activity was not affected by this high salt concentration but the protease activity was. Thus this analysis was completed on brines that had been desalted, with PD-10 desalting columns, according to instructions from the kit manufacturer (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) using dH2O as an equilibration medium, prior to the assay. Both analyses were conducted in triplicate.

The peroxidase activity was measured by a semi-quantitative analysis, according to Osman et al. [26] with some modifications. In short, 50 µL brine (centrifuged at 13 684 g for 3 minutes) and 850 µL phosphate buffer (0.5 mM, pH 6.6) were mixed and used as blank. Fifty µL quercetin (1 mM in dimethylformamide (DMF)) and 50 µL H2O2 (20 mM) were added and the absorbance at 370 nm was measured immediately (Shimadzu UV-1800 Spectrophotometer, Holm&Halby, Denmark). Results were expressed as ΔAbs/min/ml.

The protease (caseinolytic) activity was measured on the desalted brines using a green fluorescence [27] EnzChek Protease kit (Life Technologies, Denmark). In short, 100 µL brine (centrifuged at 13 684 g, 3 minutes) were mixed with 100 µL working solution (10 mM Tris-HCl, pH 7.75, 25°C) and the absorbance was measured at excitation/emission of 485/530 nm at one minute interval during an hour at 25°C (Spectra Max Gemini, Molecular Devices, UK). Results were expressed as ΔRFU/min/ml (RFU being Relative Fluorescence Units).

**Statistical analysis**

The measurements were carried out in triplicate on two independent aliquots of brine unless otherwise stated. The results are given as mean values ± absolute standard deviations for independent aliquots. For all statistical analysis, the GraphPad Prism® software Ver. 4.03 was used with p < 0.05. Results were compared using one-way ANOVA test with Turkey’s posttest or two-way ANOVA test with Bonferroni posttest.

**Results and Discussion**

**Proximate composition analysis**

The six different brines, VC, SC, Tsa, D-Tsa, TSp and D-TSp, obtained from the production of four different end-products, see Table 1, were characterized for their basic biochemical composition. Table 2 summarizes the pH, dry matter (DM), ash, salt, iron and total fatty acid.
(TFA) content in the brines. Except for VC, the pH ranged from 5.72 ± 0.01 to 6.96 ± 0.05 in all the brines. These values are in accordance with the values found by Nielsen [10], who studied the ripening of barrel-salted herring during 189 days in which the pH-values were reported to be around 6. The pH in VC was significantly (p<0.05) lower, namely 4.03 ± 0.00, which is in accordance with earlier values found in different vinegar brines from four different brands: 3.85-4.35 [28].

The DM and ash content of the six brines were very different. TSp and TSp contained 37.88 ± 0.34 and 26.62 ± 0.09 wt% DM, respectively. However, the ash content in these two brines was identical indicating a much higher percentage of organic material in TSp compared to TSp. This cannot be explained by the differences in curing time of the herring (both ca. 450 days) but might be due to interactions between spices and proteins/enzymes which may lead to less protein leaching out into the TSp brine. SC contained 23.22 ± 0.59 wt% and 14.58 ± 0.56 wt% of DM and ash, respectively, and thus the DM content in this brine was similar to that of TSp, even though the curing time was shorter for SC: approximately 200 days vs. 450 days for the TSp. These results are in agreement with previous reports showing that both DM and protein content in brines from marinated herring changes significantly within the first 200 days of the ripening period and thereafter these leveled off [16]. The generally high DM content found in the brines was expected as it has been shown that up to 30% of the raw herring weight is lost during marinating whole carcasses and fillets [15], and thus biomolecules leak into the brine during marination. The volume and concentration of biomolecules in the brines depend mainly on the raw fish composition, additives used, processing water quality and process operations. In contrast to the three brines not containing acetic acid (TSp, TSp and SC), VC had a significant (p<0.05) lower DM and ash content; 13.16 ± 1.90 and 5.80 ± 0.46, respectively. When acid is present and the pH is lower than the isoelectric point of muscle proteins, electrostatic repulsion results in solubilization of the proteins. But when salt is added, this prevents protein solubilization (salting-out effect) and the structure “tightens up”, which results in an increased firmness of the herring muscle and dehydration [29]. Rodger et al. [30] have shown that acetic acid penetrates the herring muscle quicker than salt and together they cause an initial hardening of the tissue, while simultaneously the drop in pH will activate proteolysis which will result in subsequent tissue softening. These processes are likely taking place in VC, resulting in less pronounced leakage of proteins into the brine, observed when compared to other brines.

The brines were analyzed for their total fatty acid content, and except for VC the total fatty acids ranged from 4.01 ± 0.28 to 8.92 ± 0.38 mg/mL (Table 2). The fatty acid analysis showed that VC contained a significantly (p<0.05) higher amount of fatty acids (20.1 ± 2.5 mg/mL) compared to any of the other brines. This was expected as fatty acids are better solubilized under acidic conditions compared to pure water. Also, it seems that the two desalting brines contained the same level of fatty acids as the corresponding blood brines (TSp and TSp). The reason for this might be that a noteworthy amount of the fatty acids are transferred via the fish to the fresh water during the desalting step. It should be noted that the comparison between the four types of brines is not straightforward, as the fat content in herring can vary from 1.3% to 25.7% [31] and the fat content of the initial herring raw material used was not measured. Hence, even though herring producer prefers high-fat herring, variations occur among the supplied raw material, and between these four products which were produced from different herring batches.

The iron content was determined in the brines, and it was expected that TSp and TSp would contain the highest levels due to the presence of blood (hemoglobin, Hb) in the brine. Indeed, the level of iron was highest in these brines with 5.68 ± 1.14 mg/kg and 4.16 ± 0.20 mg/kg for TSp and TSp, respectively, and thus can be attributed to the Hb content. However, processing salt is also known to contain trace elements and can influence product quality and some of the iron might originate from the salt. The corresponding desalting brines contained only 0.75 mg/kg of iron each, which presumably are blood residues from the herring fillets together with contribution from the salt. The iron content found in SC and VC were 2.08 ± 0.46 mg/kg and 1.61 ± 0.12 mg/kg, respectively. These products are prepared directly from fillets compared to TSp and TSp and consequently were expected to contain ferly less blood and thus less iron.

Table 3 shows the protein content, nitrogen content and density of the six brines. High protein content was present in TSp and SC, with values of 56.74 ± 0.02, 48.37 ± 0.02 and 41.66 ± 0.01 mg/ml. However, processing salt is also known to contain trace elements and can influence product quality and some of the iron might originate from the salt. The corresponding desalting brines contained only 0.75 mg/kg of iron each, which presumably are blood residues from the herring fillets together with contribution from the salt. The iron content found in SC and VC were 2.08 ± 0.46 mg/kg and 1.61 ± 0.12 mg/kg, respectively. These products are prepared directly from fillets compared to TSp and TSp and consequently were expected to contain ferly less blood and thus less iron.

Table 2: pH, dry matter, ash, salt, iron and fatty acid content of the different brines.

| Brine | pH     | Dry matter content, wt% | Ash content wt% | Salt content wt% | Iron content (mg/kg) | Total fatty acids (mg/mL) |
|-------|--------|-------------------------|-----------------|------------------|---------------------|--------------------------|
| VC    | 4.03 ± 0.00± | 13.16 ± 1.90± | 5.80 ± 0.46± | 5.55 ± 0.09± | 1.61 ± 0.12± | 20.1 ± 2.51± |
| SC    | 5.82 ± 0.01± | 23.22 ± 0.59± | 14.58 ± 0.56± | 14.21 ± 0.16± | 2.08 ± 0.46± | 4.01 ± 0.28± |
| TSp   | 5.77 ± 0.02± | 37.88 ± 0.34± | 16.12 ± 0.23± | 15.71 ± 0.08± | 4.16 ± 0.20± | 8.92 ± 0.38± |
| TSp   | 5.72 ± 0.01± | 26.62 ± 0.09± | 16.12 ± 0.06± | 15.53 ± 0.11± | 5.68 ± 1.14± | 5.36 ± 0.28± |
| D-TSp | 6.32 ± 0.01± | 8.27 ± 0.07± | 5.14 ± 0.04± | 4.94 ± 0.03± | 0.75 ± 0.39± | 8.13 ± 0.54± |
| D-TSp | 6.96 ± 0.05± | 1.91 ± 0.04± | 1.02 ± 0.01± | 0.99 ± 0.01± | 0.75 ± 1.76± | 4.45 ± 0.13± |

Values are given as means (n = 3) ± standard deviations (absolute values). By columns, letters indicate homogeneous values (p < 0.05).

Table 3: Total protein content, total nitrogen and density of the six brines.

| Brine | Soluble protein mg/mL | Nitrogen wt% | Density mg/mL |
|-------|-----------------------|--------------|--------------|
| VC    | 9.34 ± 0.01±         | 0.90         | 1042 ± 11±   |
| SC    | 41.66 ± 0.01±        | 3.64         | 1145 ± 2.7±  |
| TSp   | 48.37 ± 0.02±        | 4.21         | 1149 ± 9.6±  |
| TSp   | 56.74 ± 0.02±        | 4.80         | 1182 ± 14±   |
| D-TSp | 13.16 ± 0.01±        | 1.26         | 1048 ± 5±    |
| D-TSp | 4.39 ± 0.01±         | 0.43         | 1026 ± 6.7±  |

Values are given as means (n = 3) ± standard deviations (absolute values). By columns, letters indicate homogeneous values (p < 0.05).† The protein content in wt% is calculated from the density, and all the standard deviation is below three significant figures.
mL, respectively. The desalting brines, D-TSp and D-TSa, had lower protein content with 4.39 ± 0.01 mg/mL and 13.16 ± 0.01 mg/mL, respectively, and VC had a protein content of 9.34 ± 0.01 mg/mL. The high content of proteins in Tsa and TSp is in accordance with a former study from our group [32], in which a protein content of 49 ± 2.2 mg/mL was reported from traditional barrel-salted herring brine. Further, Svensson et al. [8] and Andersen et al. [16] have both reported a protein concentrations of 50-60 mg/mL in brines from traditional barrel-salted herring ripened for 180 and 400 days, respectively. According to our knowledge, protein content for SC has not previously been reported. The protein content in VC was the lowest and this was probably due to the fact that acid denatures the proteins and thus reduces the protein solubility [29,33,34].

In accordance with the protein content, the highest nitrogen content was found in Tsa and TSp, while the lowest content was found in D-Tsa and D-TSp (Table 3). Szymczak and Kolakowski [9] showed that the amount of total nitrogen diffusing from herring meat into the brine is large and significantly increases with ripening time. The same authors also found that the loss of total nitrogen from fillets was higher than that from whole herring, most likely due to the larger exposed tissue surface. Total nitrogen and protein content were used to deduce and estimate the amount of non-protein nitrogen in our samples (values from Table 3 and a Kjeldahl factor of 6.25). The values found by Szymczak [15] were higher than the levels found in this study for Tsa and SC, which are reported here to be 4.16 g/kg and 0.78 g/kg for whole herring and fillets, respectively. As stated in the materials and methods, Tsa and TSp are placed in the brine first as whole fish and subsequently as fillets, whereas SC and VC are filleted prior to brining, and for this reason it was expected that the level of biomolecules leaking from the herring into the brine would be higher in Tsa and TSp, with leaking of blood protein first and muscle protein in the later stage. Additionally, the longer ripening period for Tsa and TSp may also explain the higher level of non-protein nitrogen in these samples [16].

The proteinaceous fractions that are extracted into the brine can be divided into free amino acids (AA), peptides and muscle proteins including sarcoplasmic and myofibrillar proteins [10]. The protein profile of the six brines is shown in Figure 1. All brines, but VC, showed protein bands between 200 and 14 kDa and heavy bands in the 50 to 35 kDa region. TSp and Tsa showed similar profiles, which also matched the respective desalting brines, D-TSp and D-TSa. SC showed a different protein profile when compared to the other brines, with an intense fragment at approximately 45 kDa. VC showed no bands on the gel which might be due to a combination of small peptides in this brine (smaller than 14 kDa) and that the acid have precipitated the proteins in the fish, thus less proteins leak into the brine. The protein pattern found in this study for Tsa is in agreement with previous published results of brines from traditional barrel-salted herring [16,17,30] and similar with that of wastewater from salted codfish [35]. Christensen et al. [17] analyzed the brine from traditional barrel-salted herring after two days ripening and found several bands between 50 and 30 kDa. Andersen et al. [16] showed that the actin band (42 kDa) was fading in the herring muscle as a consequence of protein degradation and solubilization, and simultaneously became more pronounced in the brine during ripening. A rather heavy actin band was found in all brines, except in VC, as seen in Figure 1. Andersen et al. [16] also showed that herring myosin (200 kDa) was degraded during ripening and that several protein bands, reported to be myosin fragments, simultaneously developed in the region 200 to 40 kDa. Others have reported degradation products of herring myosin at 155, 146, 138, 123, 105, 65 and 56 kDa fragments [30], some of which seem to be present in the gel presented in Figure 1, even if further confirmation is needed.

**Total amino acid composition**

Besides the chemical characterization given above, it is also of interest to investigate the amino acids present in the brines, due to the fact that some amino acids have biological activities such as antioxidant activity. Indeed, Pampanin et al. [36] showed that peptides from fish origin have interesting antimicrobial, antioxidant and immunomodulatory properties. Table 4 shows the total amino acids in the six brines divided into essential and nonessential amino acids. The amount of essential amino acids varied and was dependent on the type of brine. For example, all the brines were rich in lysine, but lysine was more abundant in D-TSp, D-TSa and VC compared to the other brines. The opposite was seen regarding the amount of valine, which was much higher in TSp, Tsa and SC compared to the other three brines. Leucine was also present in high amount in all the brines although in a lower amount in the two desalting brines (D-TSp, D-TSa). Regarding the non-essential amino acids, alanine, glycine and proline were present in higher amounts in TSp, Tsa and SC than in VC, D-TSa and D-TSp. Both aspartic acid (+ asparagine) and glutamic acid (+ glutamine) were present in high amounts in all the brines and were representing approximately 30% of the amino acids in the two desalting brines. Table 5 presents the free amino acids in the brines as essential and nonessential amino acids and as observed in the total amino acid profile, some brines had similar free amino acid profile, with grouping of Tsa, TSp and SC as well as D-Tsa, D-TSp and VC. For example, for the essential amino acids Tsa, TSp and SC were rich in lysine and threonine compared to D-TSa, D-TSp and VC which were rich in valine. All the brines, particularly VC, were rich in leucine. Regarding the non-essential amino acids, D-TSp, D-TSa and VC were rich in aspartic acid (+ asparagine), glutamic acid (+ glutamine) and serine. High amounts of these amino acids have previously been shown in the brines from barrel-salted herring [37]. Several amino acids are known to have antioxidant property, hereunder histidine, tryptophan, glycine, alanine, cysteine and lysine [38-40]. Pampanin et al. [36] analyzed small peptides (≤ 10 amino acids) from residual herring material (frame) and found that peptides showing an antioxidant activity included glycine, proline, lysine, alanine, histidine and glutamic acid. Another study performed on whole herring hydrolysates demonstrated
that antioxidant activities of the hydrolysate were associated with high amounts of glutamic acid, aspartic acid, glycine, alanine, leucine and arginine [12], which were also found in the brines of this study.

**Antioxidant activity in the brines**

In an attempt to further characterize the brines; three *in vitro* tests were performed to evaluate their antioxidant activity. The results are summarized in Table 4 and Table 5. These tests included the determination of total phenolics, DPPH scavenging activity, and reducing power. The brines exhibited varying levels of antioxidant activity, with some showing high activity, as indicated by low scavenging capacity.

### Table 4: Total amino acid profile of the six different brines.

| Amino Acid         | TSp  | D-TSp | TSa  | D-TSa | VC  | SC  |
|--------------------|------|-------|------|-------|-----|-----|
| Histidine          | 1.11 ± 0.04 | 1.73 ± 0.27 | 1.43 ± 0.00 | 1.49 ± 0.03 | 3.17 ± 0.55 | 2.53 ± 0.74 |
| Isoleucine         | 2.76 ± 0.14 | 2.64 ± 0.89 | 3.90 ± 0.23 | 2.20 ± 0.21 | 2.47 ± 0.25 | 3.67 ± 0.16 |
| Leucine            | 7.47 ± 0.56 | 5.57 ± 1.01 | 8.04 ± 0.22 | 6.22 ± 0.25 | 7.81 ± 0.72 | 8.61 ± 0.88 |
| Lysine             | 9.68 ± 1.35 | 11.02 ± 0.90 | 9.85 ± 0.56 | 12.67 ± 0.64 | 14.95 ± 0.40 | 10.61 ± 0.90 |
| Methionine         | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |
| Phenylalanine      | 2.76 ± 0.34 | 2.74 ± 0.12 | 2.67 ± 0.08 | 2.45 ± 0.04 | 3.69 ± 0.11 | 3.68 ± 0.51 |
| Threonine          | 4.79 ± 0.21 | 4.06 ± 0.12 | 5.08 ± 0.06 | 3.88 ± 0.13 | 5.17 ± 0.01 | 4.87 ± 0.20 |
| Tryptophan         | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |
| Valine             | 4.31 ± 0.08 | 1.69 ± 0.49 | 5.00 ± 0.16 | 2.68 ± 0.15 | 1.49 ± 0.44 | 4.26 ± 0.64 |
| Total (a)          | 32.88 | 29.44 | 38.18 | 31.59 | 38.75 | 38.23 |

### Table 5: Free amino acid profile of the six different brines.

| Amino Acid         | TSp  | D-TSp | TSa  | D-TSa | VC  | SC  |
|--------------------|------|-------|------|-------|-----|-----|
| Histidine          | 1.32 ± 0.33 | 1.86 ± 0.55 | 1.47 ± 0.21 | 3.41 ± 1.19 | 3.78 ± 0.27 | 3.09 ± 1.01 |
| Isoleucine         | 2.70 ± 0.08 | 2.78 ± 0.28 | 3.78 ± 0.40 | 3.68 ± 0.09 | 4.03 ± 0.10 | 3.77 ± 0.13 |
| Leucine            | 7.05 ± 0.49 | 6.97 ± 0.77 | 8.28 ± 0.30 | 7.92 ± 0.19 | 11.65 ± 0.27 | 9.86 ± 0.31 |
| Lysine             | 7.46 ± 0.24 | 10.99 ± 0.50 | 7.49 ± 0.46 | 10.49 ± 0.74 | 11.45 ± 0.58 | 9.69 ± 0.24 |
| Methionine         | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |
| Phenylalanine      | 2.86 ± 0.01 | 3.89 ± 0.38 | 3.14 ± 0.03 | 4.34 ± 0.61 | 4.85 ± 0.39 | 4.77 ± 0.33 |
| Threonine          | 3.78 ± 0.47 | 5.46 ± 0.40 | 4.59 ± 0.12 | 5.72 ± 0.53 | 6.02 ± 0.27 | 4.67 ± 0.22 |
| Tryptophan         | n.a. | n.a. | n.a. | n.a. | n.a. | n.a. |
| Valine             | 4.29 ± 0.54 | 3.16 ± 0.10 | 6.32 ± 0.69 | 3.98 ± 0.59 | 2.94 ± 0.39 | 5.37 ± 0.08 |
| Total (a)          | 28.54 | 35.19 | 35.05 | 39.54 | 44.72 | 40.63 |

**Values are in % of total amino acids (last row) in the brines and given as means (n = 3) ± standard deviations (absolute values). n.a. not analyzed.**

**Antioxidant activity in the brines**

In an attempt to further characterize the brines; three *in vitro* tests were performed to evaluate their antioxidant activity. The results are summarized in Table 4 and Table 5. These tests included the determination of total phenolics, DPPH scavenging activity, and reducing power. The brines exhibited varying levels of antioxidant activity, with some showing high activity, as indicated by low scavenging capacity.
were used to determine their antioxidant activity. As seen in Figure 2, all the brines exhibited good iron chelating activity. VC had the lowest activity (∼55%), TSp reached ~70% activity, whereas the other four brines had activity between 80% and 90%. Interestingly, the desalting brines had very good iron chelating properties indicating that low concentration of low molecular weight compound present in the desalting brines might be acting as good chelators. Sannaveerappa et al. [41] reported that organic acids may play a major role in the antioxidant activity of herring press juice. Taheri et al. [32] previously fractionated the brine from traditional barrel-salted herring and analyzed the different fractions with the same assay, but found only negligible activity compared to EDTA. The higher iron chelating activity found in our study might be explained by the much higher protein and salt contents in our unfractionated brines.

The reducing power of the six brines is presented in Figure 3. SC, TSa, TSp, VC and D-TSa showed similar or higher reducing power compared to the positive control, and D-TSp showed a poorer reducing power. The graphs in Figure 3 clearly show a concentration dependency of the reducing power, a dependency that has been showed before in herring salt brine [30]. Farvin et al. [23] studied the reducing power of yoghurt peptides and reported that it was the lower molecular weight fractions (3-10 kDa and <3 kDa) that contained the compounds with the highest reducing power. Fractionation was not done in our experiment so it is not possible to conclude on molecular weight of the antioxidant fraction and its exact nature. Sannaveerappa et al. [41] showed that press-juice from herring muscle efficiently prevented lipid oxidation in Hb-enriched washed cod muscle mince; with most of the antioxidant activity being located in the <1 kDa fraction.

Figure 4 illustrates that all the crude brines had the same, or higher, ability to scavenge ABTS-radicals as 2.5 mM Trolox, with TSa and
TSp being the most potent samples. Dilution of the brines decreased the radical scavenging ability, thus showing a clear concentration dependency. The ability of protein-free fractions from herring salt brine to scavenge radicals in a concentration dependent manner has previously been shown by others [32], who showed no efficient DPPH-radical scavenging activity using this probe. This is in contrast with our results using ABTS as the probe showing that the results may be depending on the assays used.

The antioxidant effect of a hydrolysate of soybean protein was shown, by Chen et al. [42], to be mostly associated with short peptides (5-16 amino acids) that contain hydrophobic amino acids, valine and leucine, at the N-terminus, and proline, histidine or tyrosine in their amino acid sequence. In a previously published review by Freitas et al. [43] these same amino acids were, together with the sulfur containing amino acids, reported to provide antioxidant activity. All of the brines tested here contained these amino acids (Table 4). In some case the desalting brine also had very good antioxidant activity indicating that low concentration of low molecular weight compound, which are easily diffusing from the muscle to the brine, are potent antioxidants.

Antioxidants can be beneficial to human health as they may protect the body against Reactive Oxygen Species (ROS), which can modify membrane lipids, proteins and DNA and which are implicated in many diseases such as cardiovascular conditions, diabetes, cancer and Alzheimer’s. Dietary intake of natural antioxidants could be an important defense mechanism against ROS [44] and the present results show a potential of turning herring process water into value added products such as dietary supplements. The brines might also have good potential as food additives in order to prevent oxidation. The three tested antioxidant activities can, according to different mechanisms, prevent oxidation in food. Nevertheless, the classic in vitro tests for measuring antioxidant activity may not reveal the exact antioxidant potential of the brines in foods, and thus, tests in different food matrices should also be conducted [43]. Based on Figures 2-4, the most promising brines for further testing the antioxidant activity is the two blood-rich brines, TSa and TSp, together with SC and future studies will reveal whether the activity is solely in the protein-free fraction or if protein/peptides contribute to their antioxidant activity.

**Enzymatic activity in the brines**

The peroxidase activity in the brines is presented in Table 6. TSa and TSp showed a clear activity, with values of 2.20 ± 0.19 and 0.92 ± 0.04 ΔAbs/min/ml, respectively. D-TSa, D-TSp, VC and SC did, on the other hand, not reveal any peroxidase activity. It was expected to find peroxidases in TSa and TSp as these two brines are rich in blood and consequently Hb, which is known to have pseudo-peroxidase activity [45]. However, the presence of other peroxidase enzymes is not ruled out, but requires further characterization.

The general protease activities measured as caseinolytic activity

![Graph showing radical scavenging activity (OD734) in the brines. The activity of the samples is plotted against the dilution factor (0-2187). Control consisted of 2.5 mM Trolox. Error bars indicates 95% confidence interval.](image-url)

**Figure 4:** Radical scavenging activity (OD$_{734}$) in the brines. The activity of the samples is plotted against the dilution factor (0-2187). Control consisted of 2.5 mM Trolox. Error bars indicates 95% confidence interval.

| Brine | Peroxidase activity ΔAbs/min/ml | Protease activity ΔRFU/min/ml | Assay pH |
|-------|---------------------------------|------------------------------|----------|
| VC    | n.d.                            | 6.81 ± 1.86a                 | 7.49     |
| SC    | n.d.                            | 4.23 ± 0.67a                 | 7.25     |
| TSa   | 2.20 ± 0.19a                    | 11.51 ± 2.04a                | 7.25     |
| TSp   | 0.92 ± 0.04a                    | 3.75 ± 1.55b                 | 7.15     |
| D-TSa | n.d.                            | 11.24 ± 1.22a                | 7.61     |
| D-TSp | n.d.                            | 4.40 ± 1.55b                 | 7.71     |

Values are given as means (n = 3) ± standard deviations (absolute values). n.d., not detected.

**Table 6:** Peroxidase and protease activity in the six brines.
in the six brines are shown in Table 6 and show marked differences between brines. Some of the differences may be explained by differences in final pH during assaying which ranged from 7.15 – 7.71. As the brines will contain different types of exo- and endopeptidases with different pH optima [10], the final assaying pH can influence the total activity in the brine. Protease activity was highest in Tsa and D-Tsa. The fact that D-Tsa contained the same level of protease activity as Tsa was very surprising even though the final assay pH was slightly higher for D-Tsa (7.61) compared to Tsa (7.25). It is noteworthy that desalting for one day in fresh water will provide the same level of protease activity as nearly two years in the blood brine. This indicate that only a part of the proteases has been extracted during ripening and that considerably amount of enzymes is still present in the tissues and can be extracted during desalting. The higher activity in Tsa compared to SC and VC was expected due to presence of visceras in the herring during ripening and thereby the contribution from intestinal proteases. It was therefore also noteworthy that Tsp contained only approximately 1/3 of the activity of Tsa. Since Tsa and Tsp were both prepared from whole herring, enzymes from the intestinal and digestive track are expected to leak out into the brine, and we would have expected similar enzyme activity in these two brines. The main difference between Tsa and Tsp are the spices and it cannot be excluded that spices contain compounds that can have protease inhibiting properties, and that some of the added spices are influencing the activity of the proteases in the brine. However, it is also possible that the difference is mainly caused by batch variation. The analyzed brines are from a conventional industry, thus the herrings that resulted in Tsa and Tsp are not caught at the same place or processed at the same time, thereby not from the same raw material, and thus might represent different digestive enzyme activity. We have analyzed samples representing a snapshot of the actual industry and not from controlled ripening experiments. This is also supported by protease activity measured in D-Tsp which was at the same level as Tsp and thereby exhibited the same pattern as for Tsa and D-Tsa. This also support that proteases can be extracted during the desalting process. The protease activity in VC was 6.81 ± 1.86 ΔRFU/min/ml, and thus, the second highest. This moderately high activity may be explained by the low pH in the VC brine (4.03) which might activate some muscle proteases to a greater extent than the higher pH in the other brines (5.72 – 6.96), especially SC where the activity also originates from muscle proteases. However, to elaborate more specifically on the exact nature of the exo- and endopeptidases responsible for the protease activity in the different brines, more specific investigations are needed [10,46].

This study has shed light on the production of four different marinated herring products and the enormous amounts of process water generated during this production. These brines are currently discarded as waste prior to packaging of the end-product. The chemical characterization of the six brines revealed that they are very rich in dry matter, salt, protein, non-protein nitrogen, iron and fatty acids. In fact, with more than 200 L brine per 100 kg herring produced, this could sum up to 4 kg of lipid and more than 11 kg of protein. In addition, our investigation has demonstrated that there is a huge potential for extracting compounds of high marked value from these herring by-products, due to the antioxidative and enzymatic activity reported here. The desalting brines showed to be promising sources of antioxidants and enzymes. The use of natural antioxidant extracts to replace synthetic antioxidants in foods is currently in focus, and as such herring brine can become an interesting novel food ingredient. However, more studies are needed to further characterize the precise nature of the antioxidants and enzymes and to investigate their potential application in foods or their conversion into compounds with good commercial value.

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References

1. Smidt P (2013) Delivering food security without increasing pressure on land. Global Food Security 2: 18-23.
2. Godfray HJC, Beddington JR, Crute IR, Haddad L, Lawrence D, et al. (2010) Food security: The challenge of feeding 9 billion people. Science 327: 812-818.
3. Mahro B, Timm M (2007) Potential of biowaste from the food industry as a biomass resource. Eng Life Sci 7: 457-468.
4. Guérard F, Sellos D, Gal YL (2005) Fish and shellfish upgrading, traceability. Adv Biochem Engin/Biotechnol 96: 127-163.
5. Kim SK, Mendis E (2006) Bioactive compounds from marine processing byproducts – a review. Food Research International 39: 383-393.
6. Rustad T, Sterre I, Siltzyte R (2011) Possibilities for the utilisation of marine by-products. International Journal of Food Science & Technology 46: 2001-2014.
7. http://rubin.no/images/files/documents/varestrm_2009nettversion.pdf
8. Svensson VT, Nielsen HH, Bro R (2004) Determination of the protein content in brine from salted herring using near-infrared spectroscopy. Lebensm-Wiss U-Technol 37: 803-809.
9. Szymczak M, Kolakowski E (2012) Losses of nitrogen fractions from herring to brine during marinating. Food Chemistry 132: 237-243.
10. Nielsen HH (1995) Proteolytic enzyme activities in salted herring during cool storage. PhD thesis.
11. Ferraro V, Cruz IB, Jorge RF, Malcata FX, Pintado ME, et al. (2010) Valorisation on natural extracts from marine source focused on marine by-products: a review. Food Research International 43: 2221-2233.
12. Sathivel S, Bechtel PJ, Babbit J, Smiley S, Crapo C, et al. (2003) Biochemical and functional properties of herring byproduct hydrolysates. Journal of Food Science 68: 2196-2200.
13. Stine JJ, Pedersen L, Smiley S, Bechtel PL (2012) Recovery and utilization of protein derived from surimi wash-water. Journal of Food Quality 35: 43-50.
14. http://appasso.eurostat.ec.europa.eu/nui/submitViewTableAction.do
15. Szymczak M (2011) Comparison of physicochemical and sensory changes in fresh and frozen herring (Clupea harangus L) during marinating. J Sci Food Agric 91: 68-74.
16. Andersen E, Andersen ML, Baron CP (2007) Characterisation of oxidative changes in salted herring (Clupea herangus) during ripening. J Agric Food Chem 55: 9545-9553.
17. Christensen M, Andersen E, Christensen L, Andersen ML, Baron CP (2011) Textural and biochemical changes during ripening of old-fashioned salted herrings. J Sci Food Agric 91: 330-336.
18. AOAC, Official Methods of Analysis (2005) Salt (Chlorine as sodium chloride) in seafood, potentiometric method. AOAC International Method nr.: 981.10.
19. Lee CM, Trevino B, Chaiyawat I, Cruz IB, Jorge RF, Malcata FX, Pintado ME, et al. (2010) Valorisation on natural extracts from marine source focused on marine by-products: a review. Food Research International 43: 2221-2233.
20. Lee CM, Trevino B, Chaiyawat I (1996) A simple and rapid solvent extraction method for determining total lipids in fish tissue. Journal of AOAC International 79: 487-492.
21. Undeland I, Hultin HO, Richards MP (2002) Added triacylglycerols do not hasten haemoglobin-mediated lipid oxidation in wasted minced cod muscle. J Agric Food Chem 50: 6847-6853.
22. AOAC, Official Methods of Analysis (1995) Cludef protein in meat. Block digestion method. AOAC International Method nr.
23. Farvin KHS, Baron CP, Nielsen NS, Otte J, Jacobsen C (2010) Antioxidant activity of yoghurt peptides: Part 2 – Characterisation of peptide fractions. Food Chemistry 123: 1090-1097.
24. Farvin KHS, Baron CP, Nielsen NS, Jacobsen C (2010) Antioxidant activity of yoghurt peptides: Part 1-n in vitro assays and evaluation in ω-3 enriched milk. Food Chemistry 123: 1081-1089.
24. Oyaizu M (1986) Studies on products of browning reaction. Jpn J Nutr 44: 307-315.
25. Re R, Pellegrini N, Protegente A, Pannala A, Yang M, et al. (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology & Medicine 26: 1231-1237.
26. Osman A, Makris DP, Kefalas P (2008) Investigation on biocatalytic properties of a peroxidase-active homogenate from onion solid wastes: An insight into quercetin oxidation mechanism. Process Biochemistry 43: 861-867.
27. Jones LJ, Upson RH, Haugland RP, Panchuk VN, Zhou M, et al. (1997) Quenched BODIPY dye-labeled casein substrates for the assay of protease activity by direct fluorescence measurement. Analytical Biochemistry 251: 144-152.
28. Szymczak M, Szymczak B, Korokiewicz A, Felsiaik K, Bednarek M (2013) Effect of cover brine type on the quality of meat from herring marinades. Journal of Food Science 78: S619-S625.
29. Hamm R (1961) Biochemistry of meat hydration. Advances in Food Research 10: 355-463.
30. Rodger G, Hastings R, Cryne C, Bailey J (1984) Diffusion properties of salt and acetic acid into herring and their subsequent effect on the muscle tissue. Journal of Food Science 49: 714-720.
31. Nielsen D, Hyldig G, Nielsen J, Nielsen HH (2005) Lipid content in herring (Clupea harengus) - influence of biological factors and comparison of different methods of analyses: solvent extraction, Fatmeter, NIR and NMR. LWT 38: 537-548.
32. Taheri A, Farvin KHS, Jacobsen C, Baron CP (2014) Antioxidant activity and functional properties of protein and peptide fractions isolated from salted herring brine. Food Chemistry 142: 318-326.
33. Undeland I, Kelleher SD, Hultin HO, McClements J, Thongraung C (2003) Consistency and solubility changes in herring (Clupea harengus) light muscle homogenates as a function of pH. J Agric Food Chem 51: 3962-3968.
34. Toyohara M, Murata M, Ando M, Kubota S, Toyohara H (1999) Texture changes associated with insolubilization of sarcoplasmic proteins during salt-vinegar curing of fish. Journal of Food Science 64: 804-807.
35. Ferraro V, Cruz IB (2011) Characterisation of high added value compounds in wastewater throughout the salting process of codfish (Gadus morhua). Food Chemistry 124: 1363-1366.
36. Pampinan DM, Larssen E, Provan F, Sivertsvik M, Ruoff P, et al. (2012) Detection of small bioactive peptides from Atlantic herring (Clupea harengus L.). Peptides 34: 423-426.
37. Beaulieu L, Thibodeau J, Bryl P, Carboneau M-E (2009) Proteolytic processing of herring (Clupea harengus): biochemical and nutritional characterization of hydrolysates. International Journal of Food Science and Technology 44: 2113-2119.
38. Karel M, Tannenbaum SR, Wallace DH, Maloney H (1966) Autoxidation of methyl linoleate in freeze-dried model system. III. Effects of added amino acids. Journal of Food Science 31: 892-896.
39. Marcuse R (1960) Antioxidative effect of amino-acids. Nature 186: 886-887.
40. Marcuse R (1962) The effect of some amino acids on the oxidation of linoleic acid and its methyl ester. The Journal of the American Oil Chemists’ Society 39: 97-103.
41. Sannaveerappa T, Carlson NG, Sandberg AS, Undeland I (2007) Antioxidative properties of press juice from herring (Clupea harengus) against hemoglobin (Hb) mediated oxidation of washed cod mince. J Agric Food Chem. 55: 9581-9.
42. Chen H-M, Muramoto K, Yamauchi F (1995) Structural analysis of antioxidative peptides from soybean β-conglycinin. J Agric Food Chem 43: 574-578.
43. Fretas AC, Andrade JC, Silva FM, Rocha-Santos TAP, Duarte AC, et al. (2013) Antioxidative peptides: Trends and perspectives for future research. Current Medicinal Chemistry 20: 4575-4594.
44. Cai W, Gao QD, Peppa M, He C, Vlassara H (2002) Oxidative stress-inducing carbonyl compounds from common foods: Novel mediators of cellular dysfunction. Molecular Medicine 8: 337-348.
45. Østdal H, Davies MJ, Andersen HJ (2002) Reaction between protein radicals and other biomolecules. Free Radical Biology & Medicine 33: 201-209.
46. Reihart S (2007) Proteases. Bioactive Egg Compounds.