Food Chemistry 138 (2013) 214–219

Contents lists available at SciVerse ScienceDirect

Food Chemistry

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

Effect of alkaline pH-shift processing on *in vitro* gastrointestinal digestion of herring (*Clupea harengus*) fillets

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ARTICLE INFO

Article history:
Received 29 March 2012
Received in revised form 12 September 2012
Accepted 3 October 2012
Available online 8 November 2012

Keywords:
pH-Shift
Alkaline
Solubilisation
Precipitation
Gastrointestinal
In vitro digestion
Oxidation
Salt solubility
Protein
Isolate
Herring
Hydrolysis

ABSTRACT

The effect of alkaline pH-shift processing on herring (*Clupea harengus*) protein oxidation, salt solubility and digestibility, has been evaluated. For the latter, herring mince and pH-shift produced herring protein isolate, both raw and heat-treated, were digested using a static gastrointestinal *in vitro* model. The pH-shift process resulted in drastically lowered protein salt solubility and increased lipid oxidation while protein carbonyl formation was unaffected. Yet, no significant differences in the degree of hydrolysis (DH) were observed between mince and isolates after completed gastrointestinal digestion, something which was confirmed by a similar release of proteinaceous material <3 kDa and similar free amino acid profiles. The polypeptide profiles of digested samples however revealed that two peptides (33 and 36 kDa) were present in larger amounts in the digested protein isolate compared to the digested herring mince. The results indicate that alkaline pH-shift processing had limited quantitative influence on the gastrointestinal digestibility of herring proteins despite its negative effects on protein salt solubility and lipid oxidation.

1. Introduction

Proteins are an essential part of the human diet. Fish, eggs, milk and meat generally contain high quality protein, *i.e.*, protein with both a high digestibility and a content of essential amino acids covering human needs (WHO/FAO/UNU, 2007). Fish is a much demanded consumer product, but since the resources are limited, care must be taken to utilise both the wild fish catches and farmed fish in an optimal way. The pH-shift process (also called the solubilisation/precipitation process) can be a tool to isolate proteins from complex muscle sources such as whole fish and by-products, thereby improving their utilisation (Gehring, Gigliotti, Moritz, Tou, & Jaczynski, 2011). Good results have for example been achieved when applying the pH-shift process on herring (*Clupea harengus*), a small pelagic fish species currently largely underutilised for human consumption (Marmon, Liljelind, & Undeland, 2009; Marmon & Undeland, 2010; Undeland, Hall, Wendin, Gangby, & Rutgersson, 2005; Undeland, Kelleher, & Hultin, 2002b). In the pH-shift process the proteins are solubilised at extreme acid or alkaline pH-values, separated from bones and other insoluble matter, and then precipitated and dewatered at a pH around 5.5. The protein isolates have been shown to have good functionality for application in different food formulas (Gehring et al., 2011; Undeland et al., 2002b), and to contain sufficient amounts of all essential amino acids to cover adult human needs (Chen & Jaczynski, 2007; Marmon & Undeland, 2010). In one study, the nutritional value of pH-shift produced krill proteins was also evaluated using a rat model. Results showed the proteins to be of the same high quality as the reference protein casein (Gigliotti, Jaczynski, & Tou, 2008). So far, possible quantitative and qualitative differences in the gastrointestinal digestibility of proteins before and after pH-shift processing have however not been studied.

It is generally known that food processing both can improve and decrease protein digestibility. Induced structural changes can make the protein more or less accessible for the digestive enzymes and protein oxidation may decrease the digestibility by creating *e.g.*, S–S bonds (Sante-Lhoutellier, Aubry, & Gatellier, 2007; Santé-Lhoutellier, Astruc, Marinova, Greve, & Gatellier, 2008). Protein isolation by pH-shift processing has previously been shown to change the tertiary structure of cod myosin (Kristinsson & Hultin, 2003a), and to increase the hydrophobicity of cod and mullet muscle

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http://dx.doi.org/10.1016/j.foodchem.2012.10.035

proteins (Kristinsson & Hultin, 2003b; Mohan, Ramachandran, Sankar, & Anandan, 2007), indicating mis-folding and thus a changed tertiary structure. That the alkaline version of the pH-shift process drastically alters herring protein microstructure has recently been shown by transmission electron microscopy (Marmon, Krona, Langton, & Undeland, 2012). In fact, the improved protein functionality often seen for the isolated proteins has been partly ascribed to the induced mis-folding (Gehring et al., 2011). Also, changes in the protein sulfhydryl content (Yongsawatdigul & Park, 2004), protein salt solubility (Marmon & Undeland, 2010; Yongsawatdigul & Park, 2004) and lipid oxidation (Undeland et al., 2005) have been seen in rockfish and herring protein isolates made by the pH-shift process. These observations yield solid reasons to believe that protein digestibility might be altered following pH-shift processing.

The primary aim of this study was to elucidate possible differences in protein digestibility between minced herring fillets and pH-shift produced protein isolates made thereof. Secondarily, protein carbonyls, lipid oxidation (peroxide value and TBARS) and protein salt solubility of the herring mince and protein isolates were also to be studied. The comparison was done using a static *in vitro* gastrointestinal model, and to increase the relevance for human consumption, both raw and heat treated samples were compared. The digestibility was quantitatively evaluated by the degree of hydrolysis (DH) while qualitative differences between samples were evaluated through analyses of polypeptide profile, free amino acid profile and presence of proteinaceous material <3 kDa in digests.

2. Materials and methods

2.1. Materials

Herring (*C. harengus*) was caught in Skagerrak off the Swedish west coast. The herring was filleted and transported on ice to the laboratory where it was further processed within 56 h after catch. Pepsin from porcine gastric mucosa (1064 U/mg), Lipase from *Rhizopus oryzae* (40.8 U/mg), Pancreatin from porcine pancreas (4xUSP), porcine bile extract and bovine milk casein were from Sigma–Aldrich. All other reagents were purchased from commercial sources and were of at least reagent grade.

2.2. Sample preparation

The herring batch used consisted of approximately 150 skin-on fillets of each about 30 g. The skin was manually removed from the herring fillets, whereas the small residual bones were left in the fillets. The fillets were then minced in a meat grinder (Kitchen aid, St. Joseph, MI, USA) using a hole plate with 5 mm ø. During handling the herring and herring mince were kept on ice and the grinding process was performed in a walk-in cold-room (6 °C). The minced herring was then stored at −80 °C in plastic zip-lock bags (VWR, Stockholm, Sweden).

2.3. pH-Shift protein isolation

The alkaline version of the pH-shift protein isolation process was applied in a similar manner as was previously described by Marmon and Undeland (2010). Herring mince, typically 140 g, was thawed under running cold water and mixed with 9 parts ice-cold distilled water. It was homogenised twice for 30 s using an Ultra Turrax T18 Basic homogenizer (IKA, Taquara, RJ, Brazil) and the pH was adjusted to 11.2 using 2 M NaOH. The pH was monitored with a calibrated Hamilton double pore electrode (Bonaduz, Switzerland) coupled with a pH-metre (MeterLab PHM210, Radiometer analytical S.A., Villeurbanne Cedex, France). The homogenate was centrifuged at 8000*g* in a pre-cooled (4 °C) Avanti^{TM} centrifuge J-20 XP (Beckman Coulter, Fullerton, CA, USA) for 20 min and the supernatant collected by filtering through three layers of cotton gauze (AKLA AB, Danderyd, Sweden). The supernatant was adjusted to pH 5.4 using 2 M HCl and centrifuged at the same conditions as above. The pellet was collected and split in two portions, one which was directly used for moisture content analysis, protein salt solubility tests and *in vitro* digestion. The second part was packed in a plastic bag and stored at −80 °C in plastic zip-lock bags until analyses of total fat content, a second moisture content test, and oxidation products were carried out.

2.4. In vitro gastrointestinal digestion

Freshly produced protein isolate or thawed herring mince in amounts corresponding to 1.2 g dry weight were put in 50 ml falcon tubes. Distilled water was then added to reach a total sample weight of 12 g. As a blank, 12 ml of distilled water were used. Samples to be heated were put in a shaking water bath at 55 °C for 30 min, which was the time required for the core temperature of the sample to stabilise at 55 °C (data from pre-trial not shown). 55 °C was chosen as 52–55 °C degrees are suggested as an ideal temperature to serve fish. Following completed heat treatment, the samples were cooled under running cold tap water. Heated and unheated herring mince samples were then stored on ice overnight. Further, casein, only without heat treatment, was used as a reference protein in a control experiment. The casein protein content was then equal to that of the herring mince, and water added to the same total volume.

The *in vitro* digestion setup (Fig. 1) was a version of the method described by Svelander et al. (2010). All the samples were mixed for 20 s at 10,000 rpm using an Ultra Turrax T18 Basic homogenizer in 12 ml of electrolyte solution (50 mM NaCl, 14 mM KCl, 10 mM CaCl, 4 mM MgCl and 3 mM KH_2PO_4) to which lipase (110 U/ml) was added. Four ml were removed for analysis of DH and SDS–PAGE. To mimic the stomach conditions, 5 ml of electrolyte solution including pepsin (9000 U/ml) was added to the remaining 20 ml sample. The pH was adjusted to 4.0 using 1 M HCl and the samples were incubated for 30 min at 37 °C during shaking at 400 rpm. The pH was then adjusted to 2.0 and the samples incubated for another 30 min, after which the pH was adjusted to 6.9 using 1 M $NaHCO_3$. To each sample, 3 ml of pancreatin and bile solution (4 mg/ml and 25 mg/ml, respectively) was thereafter added to mimic the conditions of the small intestine, and the samples were flushed with nitrogen gas prior to incubation for an additional 120 min. Distilled water was finally added to reach the same

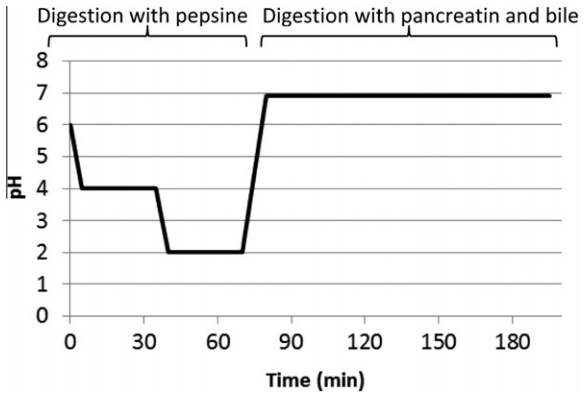

Fig. 1. Overview of the *in vitro* digestion setup.