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Nutritionally rich marine proteins from fresh herring by-products for human consumption

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ABSTRACT

Marine oils and proteins are valuable compounds, but under unfavorable conditions these components are easily oxidized and enzymatically degraded. We have designed an industrial process for producing high quality human grade proteins from fresh herring by-products. Two different processes were tested in semi-industrial scale: (i) thermal extraction to separate oil and proteins and (ii) enzymatic hydrolysis with different commercial proteases to produce fish protein hydrolysates (FPH) and separate oil. Both stick water from thermal extraction and fish protein hydrolysates, after hydrolysis, are nutritionally rich fractions and yielded approx. 18 and 25% of dry material in these fractions respectively. Neither season nor enzymes used influenced the color of hydrolysate powders, but time and temperature of the processes are important tools for controlling the color. Fishing seasons did not influence bitterness of hydrolysates and stick water samples, but both the process conditions and applied enzymes played an important role for the formation of bitterness. Insoluble fractions after both processes had significantly higher protein efficiency ratio: 3.08 and 2.93 compared to soluble fractions: 2.62 and 2.76 after hydrolysis and thermal extraction respectively. Both stick water and FPH showed antioxidative activity against both iron (15 μM of Fe^{3+}) and hemoglobin (80 ppm) induced oxidation. Herring proteins (1.25 mg/ml) were able to reduce iron induced oxidation by 50–70%, while Hb induced oxidation was reduced by 70–80% using 4 mg/ml proteins concentration.

1. Introduction

The world population is increasing every year and it is foreseen that the growth will continue the next several decades [1]. This leads to an increase in demand for high value proteins [2,3]. Existing protein sources should therefore be used more sustainably in order to ensure maximum utilization of protein for human consumption. This has driven scientists and industry to search for new environmentally sound and sustainable methods for improved utilization of existing fish catches. By-products from fish offer high protein-containing raw material, and at present they are an underutilized source of high quality protein.

Fileting processes in the fish industry receives fish of food grade, and if treated hygienically, by-products are valuable sources of marine oil and proteins which could be used for human consumption. A potential increase in the use of fish by-products for the production of valuable and active ingredients, gives a good future perspective for the marine biotechnology industry [4]. In order to achieve successful industrial implementation of processes for extraction of purified marine ingredients, two main challenges should be overcome: (1) documentation and verification of health claims should be available and (2) good sensory quality, stability and uniformity of the products must be ensured [4]. During the last decades much has been done both on scientific investigation and industrial implementation for better utilization of fish by-products [4–8]. However, only a few scientific articles deal with further processing of herring by-products, with the focus being processing of herring oil [9–13]. The protein-containing fractions make up a significant part after oil extraction and are mostly used for animal feed, but can potentially be considered as more valuable products from processing of herring by-products [14–16].

Today the herring by-products obtained from 2.2 million metric tons world catch [17] is mainly used for production of animal or fish feed and other low-value products [18,19]. From the 619,500 tons of herring which were landed from Norwegian vessels in 2011 (http://www.sildelaget.no), 238,000 tons by-products were

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produced [20,21]. This amount can give approximately 36,000 tons of proteins (assuming that by-products contain in average 15% proteins). Based on the recommended daily intake norm for proteins [22] this will satisfy the need for proteins for more than 1.5 million people per year. The main part of Norwegian herring industry is connected to Norwegian spring spawning (NSS) herring (*Clupea harengus* L.), which is a sustainable fish species (www.fisheries.no) with great importance to the North European fish industry. Currently, almost all by-products from the herring processing industry in Norway are used for animal feed and only 0.2% (mostly herring roe and milt) is used for human consumption [21]. This indicates that a considerable amount of herring by-products, containing significant amount of marine oil and proteins, is a potential source for ingredients for food production and nutraceuticals. The main part of health bringing properties of proteins is related to the composition (nutritional) properties such as amino acid composition and sequence, the size of peptide [4,23]. Since the herring by-products are rich in lipids and contain different endogenous enzymes, both lipid oxidation and enzymatic degradation start shortly after fileting. In order to produce high quality products from herring by-products, all stages of the fish processing line must be performed hygienically and by-products must be processed immediately after fileting. Thermal extraction of oil and hydrolysis are possible technological options for production of high quality proteins and fresh oil from herring by-products.

To achieve successful market penetration, produced protein should meet customer requirements. Sensory quality of protein fractions is defined by color and taste and must be acceptable to the targeted consumer groups for a successful entrance to the market [4,6,28]. Two main sensory shortcomings for the wide application of the protein hydrolysates are their color [24] and bitter taste. Color of food is one of the most important sensory properties and especially for ingredients like protein hydrolysates [24] as it could influence the color of the final product when added. Color can vary with different raw material [14], melanin pigment in raw material [25], enzymes used [26] and be influenced by dark products from oxidation of myoglobin [25]. Formation of brown pigments via the Maillard reaction [26–29] as well as process conditions [30] can also be possible sources of the development of undesirable color. The possible sources of bitter taste may be the composition of starting material or the hydrolysis process itself [31]. Identification of the sources of the bitterness and undesirable taste of hydrolysates could help to select optimal by-product composition and adjust and control processing conditions to prevent or reduce undesirable taste in protein-containing fractions. Better sensory properties would allow the use of hydrolysates in several applications, as their good functional properties [32] and high nutritional value [33,34] are well documented. In addition to these properties, peptides derived from fish proteins have shown antioxidative properties in different oxidative test systems [32,35–42].

A mobile unit for production of oil and protein hydrolysates in pilot scale (SeaLab container, design and build by SINTEF, Norway) was used for semi-industrial production of oil and protein products from the herring by-products. It was important to show that by using industrially produced fresh by-products directly from the fileting line, it is technologically possible to obtain high quality marine oil and proteins. The evaluation of how production processes influence oil quality is presented by Carvajal et al. [43]. The main aim of this work was to design an industrial process and study the influence of process conditions on the properties of protein products obtained after processing of fresh herring by-products. We also studied the effect of seasonal variation of raw material, as well as the use of different enzymes, on the yield and composition of protein hydrolysates.

2. Materials and methods

2.1. Raw material

Fresh by-products from Norwegian spring spawning (NSS) herring (*C. harengus L*) after filet production (Grøntvedt Pelagic, Uthaug, Norway) were used for the production of oil and protein fractions. By-products consisted of backbones, skins, heads and viscera. The by-products were used within one hour after collection from the processing line. The herring was caught in the Norwegian Sea. Two catches from two different seasons were used for the production. The first batch was made of by-products from herring caught in February (F) 2010 (lean herring) and second one – from herring caught in November (N) 2010 (fat herring).

2.2. Enzymes and chemicals

Papain (powder on maltodextrin, 500 TU/mg) and Bromelain (powder on maltodextrin, 400 GDU/g) (both from Enzybel Intl.s.a., Belgium) and Alcalase® 2.4L FG (Novozymes A/S, Bagsvaerd, Denmark) were used for the hydrolysis. Alcalase® 2.4L FG was kindly provided by Novozymes and comply with the recommended purity specifications for food-grade enzymes given by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and the Food Chemicals Codex (FCC) [44,45].

Methanol, chloroform, formaldehyde, hexane, diethylether, and formic acid (Merck, Darmstadt, Germany) were used for the chemical analysis. All chemicals used were of reagent grade.

2.3. Semi-industrial production of herring oil and protein fractions

A mobile unit (SeaLab container: designed and built by SINTEF Fisheries and Aquaculture, Norway) was used for semi-industrial production of oil and protein products from fresh herring by-products. The transportable unit is built by SINTEF to perform production tests on factory sites with fresh by-products. The production capacities of the unit are 1000 kg by-products/h for thermal extraction and 400 kg by-products/h for hydrolysate production by proteolytic hydrolysis. Various process configurations can easily be tested in semi-industrial scale by the mobile unit. Two technological processes were tested during these experiments: (i) thermal extraction performed at different temperature, (ii) hydrolysis process with different commercial proteases added.

2.3.1. Production of herring oil and protein fractions by thermal extraction

Fresh herring by-products obtained in February were transferred to an industrial meat mincer (disk hole diameter 10 mm) within one hour after fileting. The mincer was connected directly to a pump which fed the minced material into a closed continuous process at constant rate. The minced material was heated while passing (within 5 min) in a scraped surface heat exchanger (Votator, Waukesha Cherry-Burrell) to selected temperature (60, 70, 80 or 90 °C) to study the effect of extraction temperature on the quality of products. The heated material was directly delivered to the tri-canter (Flottweg Separation Technology, Germany), where the oil, stick water (water phase), and sludge (solid phase) were separated. Samples from all fractions were collected and frozen. Based on the results from the temperature experiment in February, 70 °C was chosen as reference and used as extraction temperature in November experiments. Schematic flow of the thermal process is presented in Fig. 1.