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Sensory and surface-active properties of protein hydrolysates based on Atlantic salmon (*Salmo salar*) by-products

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ABSTRACT

Sensory and surface-active properties of protein hydrolysates from Atlantic salmon by-products were compared based on enzyme specificity and molecular weight (MW) distribution at three levels of degree of hydrolysis (DH). The endopeptidases used were Alcalase 2.4L, Promod 671L and Protex 7L. The surface-active properties of the hydrolysates were assessed based on critical micelle concentration (CMC). Hydrolysate hydrophobicity was evaluated based on 2-butanol partitioning as a measure of hydrophobic peptide fraction (HPF). Principal component analysis was used to visualize the relationship between the sensory, surface-active and chemical properties of the hydrolysates. DH, MW distribution and enzyme specificity were all important for the formation of bitter taste and surface-active properties of the hydrolysates. High CMC, i.e. poor surface-active properties, was related to high DH and peptides based on Promod 671L. High bitter taste was positively correlated to peptides of less than 2000 Da and high HPF, DH and protein recovery (PR). Alcalase 2.4L produced significantly more bitter peptides compared to Promod 671L and Protex 7L. A reference sample, made by heat coagulation (>90 °C) and centrifugation of the raw material gave overall superior sensory and surface-active properties, but low PR.

1. Introduction

The world fisheries and fish farming industries generate large amounts of food-grade by-products after the primary manufacturing of edible products (filets, chops, mince etc.). By-products from fish processing include backbone, head, tail, viscera, blood and cut-offs, and typically constitute about 50% of the fish round weight [1]. Based on present industrial practice most of the by-products are either discarded or used within feed applications by processing to fish silage, fishmeal and oil [2]. The frame (head and backbone) contain considerable amounts of proteins, lipids and minerals with potential for higher-value food applications [3]. A promising industrial approach for the utilization of marine by-products is the manufacture of water-soluble fish protein hydrolysates (FPH) by use of exogenous proteases. This will give an increased yield of solubilized proteins due to reduced molecular weight and an increase in the number of ionizable groups [4]. However, the sensory and surface-active properties of the resulting peptides will change compared to the native protein due to release and exposure of hydrophobic amino acids and moieties. Alternatively, direct heat coagulation and mechanical separation can be used to extract a soluble fraction. This is the common practice in the fishmeal industry and gives presscake and fish solubles (stickwater) that is combined and dried to make fishmeal [5]. The obtained fish solubles contains free amino acids, small water-soluble proteins, such as sarcoplasmic proteins, and solubilized gelatin [6]. The degree of hydrolysis (DH) is only influenced by endogenous protease activity and represents the lowest level obtainable based on a specific substrate, however, with the penalty of a low yield of solubilized protein. Regardless of processing method, a neutral as possible taste will be a prerequisite for the commercial manufacture of a soluble fish protein used in human nutrition.

One of the main challenges in the manufacture of a food grade FPH is the formation of bitter and unpalatable tastes. Resulting peptide amino acid composition, hydrophobicity and chain length are all factors influencing the formation of bitter taste [7–12]. In general, bitterness is intensified when the overall hydrophobicity of the peptide molecule is increased. It is generally accepted that the formation of bitter taste is ascribed to short peptides of less than 10 amino acids [9]. Such small peptides are more rigid and does not possess secondary structures that may mask the hydrophobic side-chains [10]. Both presence and position of hydrophobic amino acids in the peptide chains, along with low molecular weight (MW) and spatial structure have been suggested as important for the for-

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mation of bitter taste [9]. However, the formation of bitter taste from peptides and amino acids is highly complex and involves at least six different taste receptors [13]. The sensory properties of a FPH is further complicated by the contents of mineral salts, lipids and lipid oxidation products, trimethylamine (TMA) etc. that might contribute to the overall taste sensation [14,15].

In addition to a palatable taste, it is desirable that the FPH possess good surface-active properties to contribute as an emulsifier or foaming agent in food formulations. The molecular properties relevant for foaming and emulsifying are in both cases related to the amphilicity, solubility, amino acid composition, MW and segmental flexibility of the peptides [4,16,17]. Peptides have amphiphilic properties due to the presence of both hydrophobic and hydrophilic amino acids and may be adsorbed at an interface with a decrease of the surface tension. The reduction of the surface tension between two phases, water and oil or water and air, is important for the formation of emulsions and foams, respectively [16,18,19]. Hydrolysis of proteins gives smaller and more flexible peptides that can spread and unfold more easily at an interface compared to the intact protein. However, a high DH usually give peptides with poor surface activity and limited emulsifying and foaming properties [20–22], presumably due to reduction of amphilicity, secondary structures and segmental flexibility.

The physicochemical properties of a protein hydrolysate are in most cases reported based on emulsifying and/or foaming properties [20,22–25]. However, most of these studies have used different and less standardized analytical protocols, making comparison of results difficult. Alternatively, the surface-active properties of protein hydrolysates may be assessed based on measurement of the critical micelle concentration (CMC), defined as the threshold concentration required for self-aggregation of the molecules [26]. The use of ^{1}H NMR for CMC determination is a well-established method that relies on the sensitivity of chemical shifts on molecular aggregation [26–28]. This phenomenon has proved a convenient measure of aggregation in complex systems with multiple constituents [28], and is regarded better suited for the complex FPHs compared to more conventionally methods, such as surface tension and dynamic light scattering. To our knowledge, no studies assessing the CMC of unmodified food protein hydrolysates as a function of DH or peptide size distribution have been published in the scientific literature.

The choice of protease and hydrolysis conditions may have great impact on peptide MW distribution, amino acid sequence, hydrophobic/hydrophilic balance and resulting sensory and physicochemical properties [21,22,29]. The enzyme specificity is a key factor influencing both number and location of peptide bonds being hydrolyzed and may be an important tool in the production of protein hydrolysates with well-defined properties, i.e. low bitter taste and low CMC. The objectives of the present study were (1) to assess and compare fish solubles based on direct extraction and after enzymatic hydrolysis of the substrate, (2) evaluate the effects of endopeptidase specificity, DH and MW distribution on the sensory, chemical and surface-active properties of FPH, (3) reveal relationships between the assessed properties and (4) to evaluate if combined low bitter taste and low CMC can be obtained based on the tested processing conditions. The results can be used to improve our understanding on how MW distribution and type of enzyme influence these properties and possibilities and limitations in the development of food grade FPH products.

2. Materials and methods

2.1. Materials

Food grade farmed Atlantic salmon (*Salmo salar*) heads and backbones were provided by Sotra Fiskeindustri AS, Sotra, Norway. The raw material was coarsely precut using a Comitrol® Processor Model 1700 (Urchel Laboratories Inc., Valparaiso, IN), vacuum packed in sealed plastic bags and stored at −30 °C. The endopeptidases used were Alcalase 2.4L (Novozymes, Bagsværd, Denmark; EC 3.4.21.62), Promod 671L (Biocatalysts, Cardiff, UK; EC 3.4.21X/3.4.24X) and Protex 7L (Dupont, Wilmington, DE; EC 3.4.24.28). All solvents and reagents for the chemical analyses were of analytical grade.

2.2. Chemical analysis

Nitrogen was analyzed by the Kjeldahl method (ISO 5983-2) [30]. pH was measured using a SS20 Seven Compact pH meter (Mettler Toledo, Columbus, OH) at ambient temperature. Peptide size distribution was measured by HPLC size exclusion chromatography using a Superdex Peptide 10/300 GL (I.D. 10 mm × 300 mm) (GE Healthcare, Uppsala, Sweden) column (measuring range 200–20 000 Da) as previously described [6]. Free amino acids were measured by HPLC using Waters Pico-Tag method and UV-detection at 254 nm [31].

2.3. Enzymatic hydrolysis

Precut salmon heads and backbones were thawed in water bath at 20 °C for 15 min. The substrate was added water (1:1 (w/w)) and transferred to a modified R10Bear Varimixer (A/S Wodschow & Co. Brøndby, Denmark) with continuous stirring (20 rpm). The mince was equilibrated at 50 °C before enzyme addition. The enzymatic hydrolyses were run to reach DH of approximately 17%, 25% and 30%, based on initial kinetic trials. The enzyme activity was terminated by heating the sample to >90 °C in a microwave oven and kept at this temperature for 10 min. The samples were cooled to <40 °C and separated in a Sorwall RC5C centrifuge (13200*g*, 20 min). The water phase was collected, filtrated through a T-2600 filter (Mall Corporation, East Hills, NY) to remove any residual particles and frozen (−30 °C) in aluminum containers prior to further analysis. An unhydrolyzed reference sample was produced by direct heat coagulation of the raw material (90–95 °C for 10 min) using a microwave oven followed by cooling, centrifugation and collection of the water-soluble phase according to the above protocol. All experiments were performed in duplicates.

2.4. Degree of hydrolysis and protein recovery

The *o*-phthaldialdehyde (OPA) reagent was prepared according to Nielsen et al. [32]. The Leucine standard was prepared by dissolving L-Leucine in water (0.13 mg/mL). Hydrolysate samples were diluted (10 mg/mL) with water and the OPA assay carried out by the addition of 3 mL OPA reagent to 400 μL sample (or standard). The samples were equilibrated for 3 min before measurement of absorption at 340 nm using distilled water as reference.

Protein recovery (PR) was determined based on the ratio between protein in the water phase to that in the original substrate:

$$PR = \frac{\text{Protein in the water phase}\,(g)}{\text{Protein in the original sample}\,(g)} \times 100\% \quad (1)$$

Based on the duplicate measurements, the standard deviation for DH and PR were estimated to be 0.3% and 1.7% respectively.

2.5. Critical micelle concentration

Hydrolysate samples were filtrated using Amicon Ultra Centrifugal Filter Unites (Millipore) with 100 kDa cutoff at 4000*g* for 30 min in a Jouan C3i centrifuge (Thermo Scientific, Waltham, MA). The filtrate was collected and 0.2 mL/g D_2O (99.9%, Sigma Aldrich) added to achieve deuterium lock. ^{1}H NMR spectra were recorded