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Original Article

Utilisation of fish industry residuals: Screening the taurine concentration and angiotensin converting enzyme inhibition potential in cod and salmon

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ABSTRACT

Fillet and residuals of farmed Atlantic salmon (*Salmo salar*) and wild caught cod (*Gadus morhua*) have been analysed for taurine concentration and angiotensin converting enzyme (ACE) inhibitory effect. With respect to taurine concentration, the residual fractions showed great variations, with the lowest concentration found in cod eye, 0.8 mg/g, and salmon fillet muscle, 0.7 mg/g, while for both cod and salmon the highest concentration was found in the heart with 8.3 mg/g and 5.6 mg/g, respectively. For the analysis of the ACE-inhibitory effect, the fractions were subjected to a simplified gastrointestinal digestion to produce hydrolysates which were then subjected to an ACE (1 mU) inhibitory assay to find the inhibition coefficient, $IC_{50\%}$. The lowest ACE-inhibitory effect was found in cod bile with an $IC_{50\%}$ at 7.6 μg/mU. Also the skin from both cod and salmon showed a low effect with 6.3 μg/mU and 6.0 μg/mU, respectively. For both the species the highest inhibitory effects were found in hydrolysates from the fillets, with 1.6 μg/mU and 2.2 μg/mU, respectively. Lesser used residuals, like organs connected to the cardiovascular system, such as heart, kidney and gills, with high taurine concentration and medium ACE-inhibitory effect, may be interesting as raw materials for niche products.

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1. Introduction

Annually, 30 million tons of seafood waste is dumped worldwide. When filleting salmon as well as cod, 40–60% of the biomass is either discarded or utilised in low-value feed products. Research and modern processing techniques may prevent underutilising this valuable biomass. Seafood residuals may contain unsaturated essential fatty acids, such as EPA and DHA, and proteins of high biological value including physiologically beneficial amino acids and peptides. Also vitamins, antioxidants and minerals are present in this material.

One predominant compound in marine raw material is the amino acid taurine (Spitze et al., 2003). Taurine, 2-aminoethanesulphonic acid, is a bioactive component known to have several positive effects on the cardiovascular system and to play an important role in many physiological processes (Niittynen et al., 1999; Bouckenooghe et al., 2006). Although commonly used in the Far East, the potential advantages of dietary supplementation of taurine have not been recognised in the west (Stapleton et al., 1997). Taurine is almost absent in plants, and in general, terrestrial animals contain less taurine than marine animals.

Angiotensin converting enzyme (ACE) converts the inactive decapeptide angiotensin I to a potent vasoconstrictor, the octapeptide angiotensin II in the renin–angiotensin system (RAS). This enzyme also inactivates bradykinin, which has a vasodilating action in the kallikrein-kinin system (KKS). Since the discovery of ACE by Skeggs et al. (1954), several synthetic ACE-inhibitory drugs have been designed for the treatment of hypertensive patients. By inhibiting ACE, both the RAS and KKS system are affected resulting in a lowered blood pressure. Food also carries a potential for ACE inhibition. It has been documented that peptides derived from the digests of various foods, both plants and animals, possess potent inhibitory activity against ACE (Hong et al., 2008). Kawasaki et al. (2000) showed, with no adverse side effects, an antihypertensive effect of peptides derived from sardine protein hydrolysates on mild hypertensive subjects.

Although utilising residuals of high biological value as animal feed may enhance the health of the animal, a greater potential, both economically and for human health, lies in functional foods and the health market. Thus, the objectives of this study were to document tissue values of taurine and ACE-inhibitory activity in fish industry residuals and to give insight into which fractions to choose for possible utilisation. For a comparison of ACE-inhibitory

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activity, a commercial product based on bonito called Katsuobushi oligopeptide (K.O.) was analysed.

2. Materials and methods

2.1. Products

The species utilised were farmed Atlantic salmon (*Salmo salar*) and wild caught Norwegian arctic cod (*Gadus morhua*). All fish were sampled in northern Norway during the spring and summer seasons. Fresh fish were divided within 12-h post mortem into the following fractions: muscle, skin, frame, brain, eye, heart, gill, kidney, stomach and/or intestine, pyloric caeca, bile, liver, spleen, sperm, eggs and blood. The muscle was homogenised from full-length fillets as it may vary caudally (Larsen et al., 2007). The skin samples were taken from the area covering the fillet. The different fractions were homogenised as intact as possible, i.e. eye and gallbladder with its fluids, brain including the optical centre, gill including the arch and the kidney carefully scraped from the backbone. Hearts were pooled and so was blood from different specimens. All samples were homogenised with a hand-held food processor and kept frozen at −50 °C in airtight containers prior to analyses. The K.O. is a dry powder produced by Nippon Supplement Inc. and was kept in its closed packets prior to analysis.

2.2. Free amino acid analysis

After thawing, the samples were homogenised once more to include thawing water. Approximately 2 g were added to 0.25–1.0 mL of the 20 mM internal standard norleucine, followed by the addition of 3.5% sulphosalicylic acid (SSA) to a final concentration of 3.1% SSA. Samples (total volume 22 mL) were homogenised at room temperature with an Ultraturrax T 25 basic (IKA Werke GmbH, Staufen, Germany) for 15 s at 17,500 rpm and centrifuged at 15,000 × *g* for 10 min. The supernate was then centrifuged at 15,000 × *g* for 3 min followed by dilution 1:5 with lithium citrate buffer and submitted to amino acid analysis.

The samples were analysed using a Biochrom 30 amino acid analyser (Biochrom Limited, Cambridge, UK) with a lithium citrate equilibrated column and post-column derivatisation with ninhydrin.

2.3. ACE inhibition analysis

The ACE inhibition analysis was conducted in three steps starting with a simplified gastrointestinal digestion, containing pepsin, chymotrypsin and trypsin to hydrolyse the proteins into peptides. The hydrolysates were applied to an enzyme assay where ACE produced hippuric acid (HA) from the substrate hippuryl-histidine-leucine (HHL). The HA concentration was revealed by quantitative HPLC analyses and a decreased concentration was correlated with inhibitory effect.

2.4. In vitro gastrointestinal digestion

Coarsely ground samples (12.5 g ww) were mixed with four parts of water and adjusted to pH 2 with 1 M HCl to simulate conditions in the stomach. The dry K.O. powder was added to nine parts of water. The incubation was initiated with the addition of 50 mg pepsin (Sigma P 6887) and was kept at 37 °C for 2 h on a magnetic stirrer. To simulate the small intestine, the pH was adjusted to 6.5 with 1 M NaOH and α-chymotrypsin (Sigma C 4129) and trypsin (Sigma T 1426) (both 50 mg) were added followed by 2.5 h of further incubation. After incubation, the solution was immediately frozen by liquid nitrogen followed by freeze drying until the samples consisted of less than 4% water. The freeze dried samples were finely ground in a mortar and kept frozen prior to analysis. The salmon samples were rich in fat and underwent a fat removal step to avoid introducing too much fat into the ACE-inhibitory assay. This was done by mixing the hydrolysed samples in two parts of water and vortexing it for 1 min followed by centrifugation at 15,000 × *g* for 10 min. The top layer, consisting of fat, was discarded and the sample was once again freeze dried.

2.5. ACE-inhibitory assay

ACE-inhibitory activity was determined based on the method reported by Cushman and Cheung (1971) by measuring the end product hippuric acid (HA) from the enzymic reaction between ACE (Sigma A 6778) and the substrate HHL (Sigma H 1635). A 250 μl volume with 2 mM HHL in 100 mM sodium borate buffer, pH 8.3 was preincubated at 37 °C for 10 min with 50 μl tissue samples. The tissue sample series contained samples weighing 5 μg, 10 μg, 20 μg, 40 μg, 60 μg and 100 μg in 100 mM sodium borate buffer. The samples were made in triplicate. Incubation for the inhibitory assay was initiated by adding 50 μl of ACE (10 mU) and was carried out on a shaker at 37 °C for 30 min in 48-well microtiter plates adding up to a final volume of 350 μl. The enzymic reactions were stopped by the addition of 430 μl 1 M HCl.

2.6. HPLC

Quantitative HPLC analyses were performed on a Waters 2695 Separations Module equipped with a Waters 2996 photodiode array detector (Waters Corporation, MA, USA), using a Symmetry-Shield reverse phase C-18 Intelligent Speed column (Waters, 20 mm × 3.0 mm id, 3.5 μm). The HA was eluted at ambient temperature using a mobile phase that consisted of 0.05% aqueous trifluoroacetic acid and HPLC-grade acetonitrile. A 2 min linear gradient was used, beginning after 0.5 min isocratic elution with 5% acetonitrile and ending with 90% acetonitrile. The flow-rate was maintained at 2 mL min^{-1} and the UV absorption was measured at 228 nm. The concentration of ACE inhibitors acquired to inhibit 50% of ACE activity was defined as the $IC_{50\%}$ value.

2.7. Water content

The water content was determined by drying approximately 10 g minced sample at 105 °C to constant weight using a Termaks Laboratory drying oven (Termaks, Bergen, Norway).

2.8. Statistical analysis

Values are presented as mean ± standard deviation (SD). Statistical analysis was performed with SPSS 14.0 (SPSS Inc., Chicago, IL, USA). Significant difference between products was calculated using analysis of variance (ANOVA) with post hoc comparison using the Tukey test. Differences between cod and salmon were evaluated using a *t*-test. Level of significance was set to $P < 0.05$.

3. Results and discussion

In Table 1, the taurine concentration in different organs and tissues of cod and salmon is presented. The organs with the lowest concentrations were cod eye, 0.8 mg/g, and salmon muscle, 0.7 mg/g, while for both cod and salmon the highest concentration was found in the heart with 8.3 mg/g and 5.6 mg/g, respectively. Taurine is believed to participate in many biological functions and the taurine concentration in the analysed fractions should vary according to the type and number of processes it is involved in. The content of taurine was relatively high in organs

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