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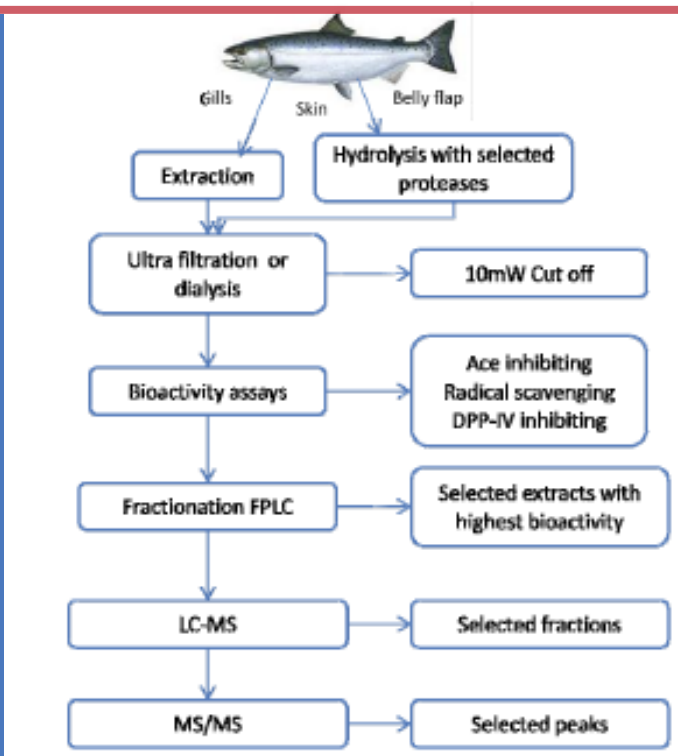
Summary of PhD thesis

Discovery and characterization of novel bioactive peptides from marine secondary products



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SUMMARY

There is an increasing interest in bioactive peptides from marine secondary products, as they offer a great potential for incorporation into functional food and for medical purposes. Bioactive peptides from marine sources have been found to display a wide range of physiological functions including antioxidative, antihypertensive, antimicrobial, immunomodulatory, anticancer and diabetes 2 effects among others. However, majority of the research has been focusing on the peptides derived from hydrolysis with commercial industrial enzymes and the usefulness of these hydrolysates.

It could be interesting whether digestion of fish secondary tissue with gastrointestinal proteases generates peptides, which also have these health promoting properties either in relation to gastrointestinal digestion or as an alternative to the use of industrial proteases. Furthermore, as a bioactive defense system against the bacterial load in the water, fish is expected to possess bio-components as small peptides. It could therefore be relevant whether these naturally occurring peptides exhibit other functional and health promoting bioactive properties.

On this background the overall goal of the present PhD research was to discover and characterize novel bioactive peptides from marine secondary products. The research was divided into two more specific objectives in different parts. Part I was to investigate naturally occurring peptides for bioactivities as radical scavenging activity, Angiotensin I-converting enzyme (ACE) and intestinal dipeptidyl peptidase (DPP-IV) inhibiting properties and protease inhibiting activity in tissue of secondary products such as gills, belly flap muscle and skin from salmon (*Salmo salar*). This was conducted in extracts from untreated and heat-treated tissue by using *in vitro* assays. Furthermore, if any detected, an aim was to characterize the corresponding candidate bioactive molecules. Part II was to investigate peptides in hydrolysates from salmon (*Salmo salar*) belly flap muscle and skin generated by gastrointestinal proteases for radical scavenging activity, DPP-IV and ACE inhibiting properties. Furthermore it was the aim to study the stability and mechanism of the muscle hydrolysates towards ACE and DPP-IV activity. Also, the corresponding candidate bioactive molecules, - if any, in selected hydrolysates should be characterized.

For the naturally occurring peptides investigated in part I, radical scavenging activity was detected in <10 kDa extracts of gills, belly flap muscle and skin with EC_{50} values of 39, 82 and 100 µg/mL, respectively. No ACE and DPP-IV inhibiting activity could be detected. Mass spectrometry analysis of dominating compounds in active fractions from size exclusion chromatography showed that families of related compounds were found in several fractions from different tissues but most pronounced in gills. One family was defined according to content of a specific amino acid sequence (PW). Three families were defined by the m/z value

of the smallest compound reported in each family (219, 434 and 403). The three latter families did not contain standard unmodified amino acids, indicating peptides with modified amino acids or other kinds of molecules.

For the peptides in the hydrolysates generated by gastrointestinal proteases investigated in part II, analysis of <10 kDa hydrolysates showed that gastrointestinal proteases generated peptides with clear radical scavenging activity and DPP-IV and ACE inhibiting activity as well. Hydrolysates from pepsin digestion exhibited the lowest EC_{50} values for radical scavenging activity and ACE inhibition, whereas EC_{50} increased in hydrolysates after subsequent digestion with pancreatic and mucosal proteases. Interestingly, EC_{50} values for the DPP-IV inhibition were hardly affected by sequential digestion. Inhibition modes for the muscle hydrolysates were both competitive and non-competitive, but prolonged incubation showed that the inhibitory properties unstable, and therefore properly digested as competitive substrates by gastrointestinal proteases.

When fractionated by size exclusion chromatography, radical scavenging activity was found in all obtained hydrolysates, though hydrolysates from belly flap muscle showed a much stronger activity compared to skin hydrolysates. DPP-IV and ACE inhibiting activity was observed in nearly all fractionated hydrolysates, only in the pepsin generated hydrolysates no pronounced (or maybe none) DPP-IV inhibitory effect was observed. This is notable, as it was not in agreement with the obtained results from EC_{50} values for the three-fold dilution curves. However, it is interesting, as it might be due to a synergy effect only present in the main hydrolysates, which vanishes when the hydrolysates are separated into fractions.

Finally, mass spectrometry analysis of dominating compounds in active fractions from size exclusion chromatography from belly flap muscle and skin hydrolysate generated from pancreatin/mucosa digestion, showed that many compounds were present in several fractions. Currently it has not been possible to identify candidate bioactive compounds responsible for a certain bioactivity, as a more thorough analysis and characterization is required as a more thorough analysis and characterization is required.

Overall, this PhD research clearly showed a potential for bioactive peptides with health promoting properties from fish secondary tissues, especially when generated with gastrointestinal proteases, both in relation to gastrointestinal digestion and as an alternative to the use of industrial proteases.