Chapter 2 Peptides and proteins

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Abstract

Seafood contains valuable proteins, and the contents and properties of these proteins are important both for the nutritional value of seafood but also for different quality aspects of the seafood and seafood products. A wide range of different analytical methods can be used to determine the amount of, the composition, and the functional properties of the seafood proteins. These can be used to follow changes taking place during processing and storage. This can be used for optimization of seafood processing and utilization.

Key words: functional properties, molecular weight, allergen

2.1 Introduction

Seafood contains high value proteins and in 2019, aquatic foods provided about 17 percent of animal proteins and 7 percent of all proteins. For 3.3 billion people, aquatic foods provide at least 20 percent of the average per capita intake of animal protein. (FAO – state of World fisheries The State of World Fisheries and Aquaculture 2022 (fao.org)). Seafood proteins also have a high nutritional value with a high content of essential amino acids. Seafood proteins and especially fish muscle proteins also have good functional properties such as water holding properties, emulsification, gelling, foaming and textural properties. For products such as surimi and fish mince products, the water holding capacity and the textural properties are important for the products produced from

surimi and fish mince. The functional properties of proteins are influenced both by fish species, season etc. but are also influenced by the processing of the raw material. Retaining the functional properties through different processing and preservation methods is therefore important to be able to make high quality products.

The proteins are also highly digestible. The amount of proteins in seafood varies with several factors, including species, size, age, sexual maturity, season and fishing ground. Since both the protein content and the properties of the proteins are highly important for the quality of the seafood raw materials. To be able to label the products as well as for quality control it is therefore highly important to have good and reliable methods to analyze the content and properties of the proteins and peptides in the seafood.

2.3 Total content of proteins

The determination of total proteins is usually carried out by determining the nitrogen content using the Kjeldahl or the Dumas method. In addition, the nitrogen content can also be determined using elemental analysis (CN analyzer) (Hjellnes et al.; 2020). These methods are described and discussed in chapter 16. Based on several cooperative studies taking into account the great assortment of methods indicates that protein determination based on nitrogen analysis for most of foods overestimates the protein content independent on specific conversion factors used.

The most reliable method to quantify proteins is to use quantitative amino acid analysis. The method involves hydrolysis of the food/raw material followed by analysis of the amount and composition of the amino acids. However, there is a need for improvement of the methods/conditions used to hydrolyse the proteins. This is further described in Chapter 16 and in Mæhre et al. (2018).

2.4 Functional properties of proteins

Functional properties have been defined as those physical and chemical properties that affect the behavior of proteins in food systems during processing, storage, preparation, and consumption (Kinsella 1982). Functional properties of proteins include water holding capacity, gelling properties, texture, viscosity, thickening, fat binding, emulsifying and foaming properties etc.

The functional properties of seafood proteins are important since they directly influence the food product including sensory and other consumer acceptability attributes. The functional properties of seafood proteins are influenced by the total physicochemical properties exhibited by the proteins while subjected to processing, consumption and storage. Changes in functional properties such as drip loss, changes in water holding capacity/water binding, cook loss, texture and gelling properties can be used to determine changes in fish and seafood during storage and processing (Hultmann and Rustad, 2002; Ofstad, et al. 1993).

The peptide and amino acid sequences of the proteins together with parameters such as pH, ions etc. determines the nature and intensity of the functionality parameters (Chalamaiah et al., 2012). Being able to determine/determination of the functional properties is important to determine the application potential of both extracted seafood proteins as well changes in the seafood proteins during processing and storage. The methods used to determine functional properties are usually not standardised and are highly lab dependent making comparison of results from different laboratories difficult. Texture can be determined instrumentally using a texture analyser (Sigurgisladottir et al. 1997).

One of the simpler methods to measure changes in proteins is to measure changes in solubility. Proteins in fish muscle can be divided into three main groups, sarcoplasmic, myofibrillar and connective proteins. These groups are based on the solubility of the proteins. The sarcoplasmic proteins are soluble in water or buffers with low ionic strength and mainly consists of enzymes. The myofibrillar proteins are also called salt soluble proteins and can be extracted in buffers with ionic strength >0.3. Concentrations of 0.6 M KCl or NaCl are often used. NaCl being an ionic salt is capable of increasing the protein solubility through the salting in process, which creates a double ionic layer augmenting the protein salt interaction (Purschke et al., 2018). The connective tissue proteins are often called insoluble proteins and to extract these alkali or acid are needed. When comparing methods and results presented in the literature it is clear that extraction methods and conditions (buffers, pH, ionic strength, ratio buffer to raw material etc) is not standardized. Since the extraction method – as well as the determination method – are important factors influencing the result, this is important to keep in mind both when setting up experiments and when comparing results from different studies.

Changes in solubility has been found to be correlated to changes in other functional properties such as water holding and textural properties. The myofibrillar proteins are susceptible to denaturation during processing and changes in the solubility or extractability of these can reflect changes during processing. This has been used to study changes during storage (ice, frozen storage) as well as heat treatment (Hultmann and Rustad 2004).

2.4.1 Water holding capacity (WHC)

Native fish muscle proteins have good water holding capacity. The water holding capacity is also important for the textural properties including juiciness and tenderness influencing the mouthfeel of the seafood products (Chan et al., 2021). Both WHC and drip loss (DL) which indicates a poor WHC, are representative parameters of freshness considering the association with water and fish muscle (Warner, 2014). The conventional methods used to measure WHC often relies on the application of an external force on the protein matrix. For determining DL, almost no force is applied and only gravitational movement of water from the proteins over a period is determined where the DL is calculated as percentage of water excluded against the original weight of the sample (Komoro et al., 2022). The external application of force used to determine WHC is by mechanical forces like compression and centrifugation. Filter paper wetness (FPW) is a simple technique used in compression, where a sample is compressed between filter papers which highly associates with DL (Mallikarjunan, 2016). In centrifugal method, a force of either lower g (200-800) utilising 2 to 15 g of sample or a higher g (5000-40000) utilising 1-20 g sample is used which measures the capacity of the sample in retaining the moisture contained after the process (Varmbo et al., 2000). The low g centrifugation method, which retains the structural integrity of the muscle tissue is largely preferred for analysing seafood WHC (Skipnes et al., 2007). Another method of using an external force is applying temperature which measures the effect of thermal process on the water holding capacity of the protein matrix when subjected to denaturation (Skipnes et al. 2007). Measurement of thaw loss after freezing is also a methodology used for analysing the WHC of the protein matrix when subjected to low temperature preservation (Bowker, 2017). The liquid loss is generally used to calculate WHC and is ultimately expressed in % through determining the weight difference of the sample before and after the liquid is separated through centrifugation (Sun et al., 2018).

2.4.2 Emulsifying property

Proteins can also stabilize emulsions. Emulsions are complex mixtures of two immiscible components, for example, oil-in-water formed under specific conditions like temperature and presence of elements which support the formation of such complexes called emulsifying agents.

These mixtures are highly unstable thermodynamically and has different applications like providing unique texture and flavour in food and in encapsulating, safeguarding and distribution of functional components into a food medium (Walker et al., 2015). To determine the emulsifying capacity a predetermined volume of protein with known concentration is homogenised with vegetable oil followed by centrifugation. The volume of the emulsion is determined and the emulsifying capacity expressed as ml of emulsified oil/g of protein (Kinsella, 1976). The emulsion stability can be defined as the percentage of initial emulsion remaining after a certain time (1 day at room temperature) and centrifugation (McClements, 1999).

2.4.3 Foaming properties

The foaming capacity of a food constituent like protein find application in the food industry regarding preparation of texture specific food items such as whipped cream, ice cream and bakery items which emphasizes on the volume and air occupancy (Lam et al., 2018). The foaming property of proteins are attributed to its pH affinity which results in precipitation closer to the isoelectric point (Yang and Baldwin, 2017). Moreover, the transportation, rearrangement, and penetration of protein molecules at the air-water interface determines the foaming capabilities properties (Elavarasan et al., 2014). Sathe and Salunkhe (1981) proposed methodologies for assessing the foaming capacity and foam stability of proteins. A predetermined mass of protein concentrate was distributed in a known volume of water, the mixture was homogenized and transferred to a graduated beaker for measurements. The foaming capacity is defined as the % volume increase over the initial volume.

2.4.4 Gelling properties

Gelling property is an important functional attribute which finds application in texture specific food applications. The gelling feature of seafood muscle protein is attributed to the thermal induced partial unfolding of myosin filaments in solution and further irreversible accumulation of unfolded filaments to form a three-dimensional formation trapping water, within the matrix. Fish muscle proteins can gel at low temperatures incubation for 12hrs at 0° to 4 °C or a mild thermal process will result gel formation (Sasidharan and Venugopal, 2020).

Gel preparation is the primary step in analysing the gelling properties of the protein concentrates. The gel strength of the samples is generally measured using an instrumental texture analyser at room temperature. The samples were subjected to pressure exerted through a spherical probe or plunger with 5mm diameter on a 10N load cell with a depression rate of 30-60 mm/min. The breaking force and deformation data was obtained and expressed as g and mm (Rawdkuen et al., 2009; Le et al., 2018).

2.4.5 Fat binding capacity

The fat binding capacity indicates the ability of the protein to absorb and retain lipid components within the matrix. This property is primarily linked to emulsifying capacity and may be also further influenced by bulk density, degree of hydrolysis and enzyme-substrate specificity depending on the extraction methodology employed (Villamil et al., 2017). The property finds application in meat and confectionary industry as it regulates the flavour characteristics (Taheri, 2013). Ozyurt et al., (2015) proposed a method for analysing the fat binding capacity of protein. The protein concentrates precisely weighed to 1g was dissolved in 10 ml of vegetable oil, mixed thoroughly for 5 minutes, and then centrifuged at 3000 x 9 g for 15 minutes. The weight difference was later expressed as the fat binding capacity.

2.4.6 Analysis of soluble proteins

To determine the amount of soluble proteins they need to be extracted from different matrixes in marine raw materials or in products where they may have interacted and can be bound to carbohydrates, lipids and other nutrients. Both direct and indirect analytical methodologies can be used for protein determination. Several of the most important indirect methods for protein determination in food date from the late 1800s (Dumas, Nessler's reagent, Biuret, Kjeldahl, Folin-Ciocalteau, and Dye binding) (Owusu-Apenten, 2002). The Kjeldahl and Dumas methods are based on quantification of the total organic nitrogen followed by conversion into crude protein or by a set of direct methods. The Biuret, Folin Ciocalteau and Dye- binding are methods where the proteins are chemically or physically modified for determination (colorimetric assays). These methods can be divided in to two groups: dye-binding reaction and redox reaction with proteins (ref). In the redox spectrophotometric methods, analyses are based on reaction with Folin reagent, and the following methods could be mentioned: Biuret reaction (Noll et al. 1974), Lowry protein method (Lowry et al, 1951), and bicinchoninic acid (BCA) assay (Walker, 2002). In the Biuret reaction Cu(II) with proteins in alkaline medium is reduced to Cu(I), which binds to protein forming a Cu(I)–peptide complex with purplish-violet color (Noll et al. 1974),. The same principle is used in BCA assay, where Cu(I) is detected by reaction with BCA, which gives an intense purple color (Liu et al., 2008). One of the most popular methods in this group is the Lowry protein method (Lowry et al, 1951), which is initially based on the Biuret reaction, where peptide bonds react with Cu(II) in alkaline medium to produce Cu(I). Later Cu(I) reacts with the Folin reagent. The reaction gives a strong blue color (Waterborg, 2002). The intensity of color partly depends on the amount of Tyr and Trp in samples but can also be influenced by other components such as N-containing buffer or carbohydrates [121]. The amounts of proteins in sardine determined by the Lowry method were comparable to those determined by Kjeldahl method (Noll et al. 1974). The Lowry method is suitable for protein extracts such as actomyosin, which is an important component in surimi-based products (Bradford, 1976). However, the BCA assay is shorter compared with the Lowry method (where two steps are needed), more flexible and stable in alkaline conditions, and has a broad linear range. The BSA assay can also be interpreted by the usual chemical components such as EDTA, thiols, reducing sugars, hydrogen peroxide, or phospholipids (Noll et al,, 1974, Liu et al., 2008]. The dye-binding spectrophotometric assay is based on the reaction between acid dye and positively charged amino acid residues in proteins (Noll et al. 1974). In acidic conditions, the created insoluble complexes are removed and the unbound dye is determined by measuring its absorbance. The amount of protein is proportional to the amount of bound dye. Coomassie dye in acidic conditions binds to proteins and creates complexes that influence a color shift from a maximum from 465 nm to 595 nm, using the Bradford method (Bradford, 1976). Absorbance of Coomassie dye-protein complex is measured at 595 (575–615) nm, because the difference between the two forms of the dye is greatest in this area. Within the linear range of the assay (~5–25 mg/mL), the protein amount is proportional to bound Coomassie (Bradford, 1976). This method is suitable for determination of extractability of proteins and determination of protein content in extracts (Benjakul and Bauer, 2000, Hultmann and Rustad, 2004, Søvik and Rustad, 2005, Kruger 2002). This technique is simple, sensitive, and uses shorter analysis time compared with the Lowry method. Moreover, the dye-binding assay is less affected by reagents and nonprotein components from biological samples (Aitken and Learmont, 2002)
Proteins in solution can be quantified in a simple spectrophotometric analysis by near- or far-UV absorbance (Van Camp and Dierckx, 2004; Watts et al., 2001). Absorption in the near UV by proteins depends mostly on the content of Tyr and Trp and less on the amount of phenylalanine (Phe) and disulfide bonds. This absorbance measurement is simple, sensitive, needs no reagents, and the sample is recoverable (Van Camp and Dierckx, 2004; Watts et al., 2001). Crude protein extracts or individual fractions of proteins [135] can be measured at 280 nm. Disadvantages of the method include interference with other components such as nucleic acid, which absorbs in the same wavelength region [133].

Far-UV absorption can also be used for determination of protein content: peptide bonds absorb in the area with the maximum at about 190 nm. Different proteins give a small variation in absorbance, and the method can be considered as accurate for protein determination. However, oxygen also absorbs at these wavelengths, and to avoid interference, measurements at 205 nm is used. It should also be mentioned that components such as carbohydrates, salts, lipids, amides, phosphates, and detergents interfere (Van Camp and Dierckx, 2004; Watts et al., 2001).

Choice of buffers, extraction technique and chemicals influence both yield and determination of proteins by spectrophotometric methods as the substances can interfere and also lead to overestimation of the protein content.

2.5 Immunoassays/detection of allergens

Seafood allergy is a global health issue related to food safety. There is no cure for seafood allergy so the most efficient way to prevent allergic reactions is to avoid eating food containing the allergens. This requires proper labeling. To achieve this there is a need for rapid, reliable and user-friendly methods for detection of the allergens (Li et al., 2023). The current methods can be divided into three groups, namely DNA-based, protein-based and aptamerbased detection methods (Faisal, Vasiljevic, and Donkor 2019).

The most common allergens in fish is parvalbumin while tropomyosin is considered to be the most common allergen in shellfish. Most of the protein based methods for allergen detection is based on the specific binding of antibodies to allergen epitopes. These can be divided into three main categories, namely enzyme linked immunosorbent assay (ELISA), immunochromatography assay, and immunosensor. The most widespread method for allergen detection are ELISA (enzyme-linked immunosorbent assay) methods. Immunoassays can be used to determine the amount of a specific protein in a mixture. Immunoassays are based on specific interactions of the antibody with the target antigen (the protein that should be determined) in the sample. This can for instance be used to analyse for presence of allergens (Fu et al, 2022).

ELISA presents advantages such as high sensitivity and large detection scale, and simple equipment design (Clark, Lister, and Bar-Joseph 1986). There are also convenient and reliable ELISA kits for seafood allergen detection commercially (Fernandes et al. 2015).

Some of the commercial kits do not have the ability to detect the allergenic peptides or proteins that has been formed during processing (heat denaturation, hydrolysis). Several studies have shown that these challenges can be solved (Li et al, 2023).

Immunochromatography methods are relatively rapid and simple to carry out, but can only be used for qualitative or semiquantitative analysis but not for quantitative analysis. Biosensors have several advantages, such as increased sensitivity but they are more time-consuming than other techniques. Currently, two main types of immunosensor used for seafood allergen detection have been developed, electrochemical immunosensor and optical immunosensor.

MS Antibody-based detection methods are sometimes inadequate for qualitative and quantitative allergen detection because of matrix/processing effects and epitope masking. However, MS-based detection methods can well resolve it (Lopez-Pedrouso et al. 2020). MS-based detection methods are usually used in determination and detection of food allergens, nevertheless, the workflows of MS-based methods are different with diverse aims. When determining a new allergen, MS is only

one tool to determine protein species, and it is often combined with other techniques to achieve the goals.

Protein-based detection methods, which take a certain allergen as the direct detection object, are mostly realized based on the binding ability of antigen and antibody. However, the detection accuracy is easily affected by the sample substrate, processing and other factors. As a protein-based detection method, mass spectrometry can overcome interference factors to some extent, but it is time-consuming and requires expensive equipment.

2.6 Electrophoresis and chromatography based methods

During processing and storage muscle proteins may be degraded, this is reflected in changes in molecular weight. Molecular weight distribution of proteins can be determined by different methods. In native gelfiltration chromatography, the proteins are separated based on their size and shape, that is based on their Stokes radii. The beads used for this are usually open, crosslinked three-dimensional polymer networks such as agarose, dextrans, cellulose, polyacrylamide or combinations of these materials (Owusu-Apenten, 2002). For high-pressure systems microporous silica, porous glass, or inorganic -organic composites are used. Small molecules can enter all the pores in the beads, whereas larger proteins and peptides can only enter the larger pores. This means that the proteins and peptides are separated by size and that the size can be determined by comparison with the elution volume of known standards. How the protein behaves in a column is described by the coefficient K_{av} which describes the proportion of the pores available to the molecules. $K_{av}=(V_e-V_0)/(V_t-V_0)$. V_e is the elution volume of the molecule, V_0 is the void volume of the column and Vt is the total volume of the column.

Molecular weight and changes in molecular weight during processing and storage can also be determined by electrophoresis (Hultmann et al. 2004). SDS PAGE is one of the most commonly used methods. This method uses polyacrylamide gels, and the proteins are denatured by boiling in a solution of sodium dodecyl sulfate (SDS). SDS binds to the proteins at a ratio of 1:1.4 and since SDSD is charged this means that the protein charge will be directly proportional to the molecular weight. DTT (dithiothreitol) or mercaptoethanol is often added to reduce disulfide bonds.

2.7 Peptide characterization

Analysis and characterization of peptides are important for several reasons. The first is because degradation of seafood proteins can result in formation of small peptides and free amino acids and analysis of these can be used to follow changes during processing and storage (Bauchart et al., 2007; Hultmann et al.; 2004). Increase in acid soluble peptides and free amino acids can be used to follow hydrolysis processes. Commercial protein hydrolysates show a large range of variation in degree of hydrolysis, ranging from 50% where at least half the original peptide bonds have been broken (Johns, et al. 2011). The size distribution of peptide fractions can be determined by chromatographic methods such as high performance size exclusion chromatography (HPSEC). Different column materials and detection methods are being used and to separate peptides in the size range from 7000 to 100 Da the Superdex Peptide HR 10/30 column is often used (Johns et al., 2011).

The amount of small peptides can be quantified by determining the content of acid soluble peptides. This can be done by precipitation to a final concentration of 10% trichloroacetic acid (TCA)(Bergvik et al., 2012). The solubility of proteins in TCA is determined both by size but also by hydrophobicity and values from 3-20 amino acid residues have been suggested as peptide sizes that are soluble in 10%

TCA. Precipitation in 70% ethanol has been shown to give the same concentration of soluble peptides as precipitation in 12% TCA (Rohm et al., 1996).

2.8 Protein modifications

Oxidation of proteins and this will lead to various changes in the proteins, both chemical changes on individual amino acids but also breakdown of the peptide backbone and aggregation (Hematyar et al. 2019). Because the functions of protein are very specific, oxidative modifications can cause numerous functional consequences and lead to changes in food texture, WHC, digestibility and juiciness (Baron et al., 2007). Protein oxidation may be caused directly by ROS and reactive nitrogen species or indirectly as the result of reactions with products from lipid oxidation with reducing sugars or carbohydrate (Lund et al., 2011). Protein oxidation is usually determined by analysis of increase in carbonyl groups (ref), decrease in sulfhydryl groups or formation of dityrosine (Lund et al., 2011).

Summary

Seafood contains valuable proteins, and analysis and characterization of seafood proteins is desirable both to understand the properties of the raw material but also to be able to understand how the proteins will behave during storage and processing. This may enable improving the and optimization of processes to make high quality seafood products. A wide range of methods to analyze the properties of seafood proteins exist, however, many of the methods are highly empirical especially when it comes to determining the functional properties of the proteins.

References

Aitken, A. and Learmonth, M. P., Protein Determination by UV Absorption, In The Protein Protocols Handbook, 2nd Edition, Edited by: J. M. Walker © Humana Press Inc., Totowa, NJ, 2002.

Baron, C. P.; Kjærsgård, I. V. H.; Jessen, F.; & Jacobsen, C. 2007. Protein and Lipid Oxidation during Frozen Storage of Rainbow Trout (Oncorhynchus mykiss) J. Agric. Food Chem. 55, 0 8118-8125

Benjakul, S. & Bauer, F. 2000. Physicochemical and enzymatic changes of cod muscle proteins subjected to different freeze-thaw cycles. *J. Sci. Food Agric.,* 80, 1143-1150.

Bergvik, M.; Overrein, I.; Bantle, M.; Evjemo, J.O. & Turid Rustad, T. 2012. Properties of Calanus finmarchicus biomass during frozen storage after heat inactivation of autolytic enzymes, Food Chemistry,Volume 132, Issue 1, p 209-215,https://doi.org/10.1016/j.foodchem.2011.10.058.

Bradford, M. A. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein using the principle of protein-dye binding. Anal. Biochem. 72 248-254.

Chalamaiah, M., Dinesh kumar, B**.;** Hemalatha, R. & Jyothirmayiet, T. 2012. Fish protein hydrolysates: Proximate composition, amino acid composition, antioxidant activities and applications: a review. *Food Chemistry*, 135, 3020-3038. DOI: 10.1016/j.foodchem.2012.06.100

Chan, S. T., Roth, B., Jessen, F., Jakobsen, A. N., & Lerfall, J. 2021. Water holding properties of Atlantic salmon. Comp. Rev Food Sci Food Saf. 1–22. https://doi.org/10.1111/1541-4337.12871

Chan, S.T.; Roth, B.; Skare, M.; Hernar, M.; Jessen, F.; Løvdal, T.; Jakobsen, A.J.; & Lerfall, J. (2020) Effect of chilling technologies on water holding properties and other quality parameters throughout the whole value chain: From whole fish to cold-smoked fillets of Atlantic salmon (Salmo salar), Aquaculture, 526, 735381, https://doi.org/10.1016/j.aquaculture.

Chi, CF; Cao ZH.; Wang B.; Hu FY.; Li ZR. & Zhang B. 2014 Antioxidant and functional properties of collagen hydrolysates from Spanish mackerel skin as influenced by average molecular weight. Molecules 19, 11211e11230.

Damodaran S. & Parkin K. 2017. Amino Acids, Peptides, and Proteins, In Fennema's Food Chemistry. CRC Press; Boca Raton, FL, USA.

Elavarasan, K., Naveen Kumar, V., & Shamasundar, B. A. 2014. Antioxidant and functional properties of (FPH) from freshwater carp (*Catla catla*) as influenced by the nature of enzyme. *Journal of Food Processing and Preservation,* 38, 1207e1214.

Faisal, M., T. Vasiljevic, & O. N. Donkor. 2019. A review on methodologies for extraction, identification and quantification of allergenic proteins in prawns. *Food Research International (Ottawa, ON)* 121:307–318. doi: 10.1016/j.foodres.2019.03.040.

Foh, M. B. K., Kamara, M. T.; Amadou, I.; Foh, B.M. & Wenshui, X. 2011. Chemical and physicochemical properties of tilapia (Oreochromis niloticus) fish protein hydrolysate and concentrate. *International Journal of Biology and Chemistry*, 2011, 1e15.

Fu, L., Cherayil, B.J., Shi, H., Wang, Y. & Zhu, Y. (2019). Detection and Quantification Methods for Food Allergens. In: Food Allergy. Springer, Singapore. https://doi.org/10.1007/978-981-13-6928-5_4

Gómez-Guillén, M.C.; Giménez, B.; López-Caballero, M.E. & M.P. Montero, M.P. 2011.Functional and bioactive properties of collagen and gelatin from alternative sources: a review. *Food Hydrocolloids*, 2011, 25, 1813–1827

He, S., Franco, C. & Zhang, W., Functions, Applications and Production of Protein Hydrolysates from Fish Processing Co-products (FPCP). *Food Research International*, 2013, 50(1), 289–297.

Hematyar, N, Rustad, T, Sampels, S, & Kastrup Dalsgaard, T. Relationship between lipid and protein oxidation in fish. *Aquac Res*. 2019; 50: 1393– 1403. https://doi.org/10.1111/are.14012

Hjellnes, V. H.; Slizyte, R.; Rustad, T.; Carvajal, A. K.& Greiff, K. 2020. Utilization of egg-laying hens (Gallus Gallus domesticus) for production of ingredients for human consumption and animal feed. *BMC Biotechnology* ; Volume 20 art. 22

Hultmann, L. & Rustad, T. 2002. "Textural changes during iced storage of salmon (*Salmo salar*) and cod (*Gadus morhua*)." J Aquatic Food Product Technology **11**(3/4): 105-123,

Hultmann, L. & T. Rustad 2004. "Iced storage of Atlantic salmon (Salmo salar) - effects on endogenous enzymes and their impact on muscle proteins and texture." Food Chemistry 87(1): 31-41,

Hultmann, L.; Bencze Rørå, A. M.; Steinsland, I.; Skåra, T., & Rustad, T. 2004. "Proteolytic activity and properties of proteins in smoked salmon (Salmo salar) - effects of smoking temperature." Food Chemistry 85(3): 377-387).

Jemil, I., Jridi, M.; Nasri, R.; Naourez Ktari, N.; Slama-Ben Salem, R.B.; Mohamed Mehiri, M.; Mohamed Hajji, M.& Moncef Nasri, M. 2014. Functional, antioxidant and antibacterial properties of protein hydrolysates prepared from fish meat fermented by Bacillus subtilis A26. *Process Biochemistry*, 49, 963e972.

Johns, P. W., Jacobs, W.A.; Phillips, R.R.; McKenna, R. J.; O'Kane, K.A. & McEwen, J.W. 2011. "Characterisation of peptide molecular mass distribution in commercial hydrolysates and hydrolysate-based nutritional products." Food Chemistry 125(3): 1041-1050.

Kramer R.M., Shende V.R., Motl N., Pace C.N. & Scholtz J.M., 2012. Toward a Molecular Understanding of Protein Solubility: Increased Negative Surface Charge Correlates with Increased Solubility, *Biophysical Journal*, 2012, 102(8), 1907-1915, https://doi.org/10.1016/j.bpj.2012.01.060.

Kruger,N.J. 2002,The Bradford Method for Protein Quantitation, In The Protein Protocols Handbook, 2nd Edition, Edited by: J. M. Walker © Humana Press Inc., Totowa, NJ,

Lam R.S.H. & Nickerson M.T., 2013 Food proteins: A review on their emulsifying properties using a structure-function approach. *Food Chemistry*, 141, 975–984. doi: 10.1016/j.foodchem.2013.04.038.

Lam, A., Can Karaca, A. & Tyler, R. Nickerson, M., 2018. Pea Protein Isolates: Structure, Extraction, and Functionality. *Food Reviews International*, 34(2), 126–147.

Li, J. ; Wang, H. & Cheng, J-H. 2023 DNA, protein and aptamer-based methods for seafood allergens detection: Principles, comparisons and updated applications, Critical Reviews in Food Science and Nutrition, 63:2, 178-191, DOI: 10.1080/10408398.2021.1944977

Listrat, A., Lebret, B., Louveau, I., Astruc, T., Bonnet, M., Lefaucheur, L., Picard, B., & Bugeon, J.,2016 How muscle structure and composition influence meat and flesh quality. *The Scientific World Journal*, 3182746. https://doi.org/10.1155/2016/3182746

Liu, R., Zhao, S.-m., Xiong, S.-b., Qui, C.-g. & Xie, B.-j., 2008. Rheological properties of fish actomyosin and pork actomyosin solutions, *J. Food Eng.,* 85, 173-179

Lowry, G.H., Rosebrough, R.J., Farr, A.L. & Randall, R.J. 1951 Protein measurements with the Folin phenol reagent. *J. Biol. Chem*., 193: 263–275

Lund, M.N.; Heinonen, M.; Baron, C. P. & M. Estévez, M. 2011. Protein oxidation in muscle foods: A review Molecular Nutrition & Food Research Vol. 55 Issue 1 Pages 83-95 http://dx.doi.org/10.1002/mnfr.201000453

Mbatia, B., Awuor, O. L., Kirwa, M. E., & Jackim, M. F. 2014. Antioxidative and Functional Properties of *Rastrineobola Argentea* (Dagaa) Fish Protein Hydrolysate. *Discourse Journal of Agriculture and Food Sciences*, 2(6), 180–189.

McClements D.J. Food Emulsions. 2015. 3th ed. CRC Press; Boca Raton, FL, USA.

Martins, V. G., Salas-Mellado M., & Prentice, C. 2011. Evaluation of functional properties in protein hydrolysates from bluewing searobin (*Prionotus punctatus*) obtained with different microbial enzymes. *Food Bioprocess Technology*, 2011, 4, 1399e1406.

Mbatia, B., Awuor, O. L., Kirwa, M. E., & Jackim, M. F. 2014 Antioxidative and Functional Properties of *Rastrineobola Argentea* (Dagaa) Fish Protein Hydrolysate. *Discourse Journal of Agriculture and Food Sciences*, 2(6), 180–189.

Mæhre HK, Dalheim L, Edvinsen GK, Elvevoll EO & Jensen IJ. 2018. Protein Determination-Method Matters. Foods. 2018 Jan 1;7(1):5. doi: 10.3390/foods7010005. PMID: 29301260; PMCID: PMC5789268.

Nalinanon, S.; Benjakul, S., Kishimura, H. & Shahidi, F. 2011. Functionalities and antioxidant properties of protein hydrolysates from the muscle of ornate threadfin bream treated with pepsin from skipjack tuna. *Food Chemistry*, 124, 1354e1362.

Noll, J.S., Simmonds, D.H. & Bushuk, W.C., 1974. A modified biuret reagent for the determination of protein. *Cereal Chem*., 52: 610–616.

Ofstad, R.; Kidman, S.; Myklebust, R.; &. Hermansson, A.M. 1993. Liquid holding capacity and structural-changes during heating of fish muscle - cod (gadus-morhua l) and salmon (salmo-salar). Food Structure 12(2): 163-174).

Owusu-Apenten, R. K. 2015. *Food protein analysis*. Marcel Dekker, Inc., New York, 2002.

Park, J.W. (ed.): Surimi and Surimi Seafood, 3rd edn. CRC Press, Boca Raton (2013)

Purschke, B., Tanzmeister, H., Meinlschmidt, P., Baumgartner, S., Lauter, K., & Jäger, H. 2018. Recovery of soluble proteins from migratory locust (Locusta migra-toria) and characterisation of their compositional and techno-functional properties. Food Research International, 106 , 271–279 .

Rohm, H.; Jaros, D.; Rockenbauer, C.; Riedler-Hellrigl, M.; Uniacke-Lowe, T. & Fox, P.F. 1996. Comparison of ethanol and trichloroacetic acid fractionation for measurement of proteolysis in Emmental cheese. Int. Dairy J, 6, p 1069-1077

Sasidharan, A. & Venugopal, V. 2020. Proteins and Co-products from Seafood Processing Discards: Their Recovery, Functional Properties and Applications. *Waste and Biomass Valorization,* 11, 5647–5663. https://doi.org/10.1007/s12649-019-00812-9

Shaviklo, G. R. 2006. Quality Assessment of Fish Protein Isolates Using Surimi Standard Methods; The United Nations University, fisheries training programme,

Sigurgisladottir, S., Torrissen, O. Ø.; Lie, Ø. ; Thomassen, M. & Hafsteinsson, H. (1997). "Salmon quality: Methods to determine the quality parameters." Reviews in Fisheries Science **5**(3): 223-252. DOI: 10.1080/10641269709388599

Skipnes, D. ; Østby, M. L. & Hendrickx, M. E. 2007. A method for characterising cook loss and water holding capacity un heat treated cod (Gadus morhua) muscle J. Food Eng. 80(4) 1078-1085. https://doi.org/10.1016/j.jfoodeng.2006.08.015

Smith, P. K., Krohn, R. I., Hermanson, G. T., Mallia, A. K., Gartner, F. H., Provenzano, M. D., Fujimoto, E. K., Goeke, N. M., Olson, B. J., & Klenk, D. C. Measurement of protein using bicinchoninic acid. 1985. *Analyt. Biochem.* **150,** 76–85.

Sovik, S. L. & Rustad, T. 2005 Effect of season and fishing ground on the activity of lipases in byproducts from cod (Gadus morhua). *Lwt-Food Sci. Technol.,* **38,** 867-876.

Taheri, A., Anvar, S. A. A., Ahari, H. & Fogliano, V. 2013.Comparison the functional properties of protein hydrolysates from poultry by-products and rainbow trout (*Onchorhynchus mykiss*) viscera. *Iranian Journal of Fisheries Science*, 12(1), 154e169.

Tanuja, S., Viji, P., Zybudheen A.A. & Joshy C. 2012 Composition, functional properties and antioxidative activity of hydrolysates prepared from the frame meat of striped catfish (*Pangasianodon hypophthalmus*). *Egyptian Journal of Biology*, 14, 27e35.

Tian, Y., Wang W., Yuan C., Zhang L. & Liu J. 2017. Nutritional and digestive properties of protein isolates extracted from the muscle of the common carp using pH-shift processing. *Journal Of Food Processing and Preservation,* 41, e12847.

Uluata S., McClements D.J. & Decker E.A. 2015. Physical Stability, Autoxidation, and Photosensitized Oxidation of ω-3 Oils in Nanoemulsions Prepared with Natural and Synthetic Surfactants. *Journal of Agricultural and Food Chemistry*, 63:9333–9340. doi: 10.1021/acs.jafc.5b03572.

Van Camp, J. & Dierckx, S. 2004. Proteins, In Handbook of Food Analysis, Physical characterisation and nutrient analysis, Volume I, Second edition, Revised and expanded, edited by Leo M.L. Nollet, Marcel Dekker, Inc., New York.

Villamil, O., Váquiro, H. & Solanilla, J. F., 2017. Fish Viscera Protein Hydrolysates: Production, Potential Applications and Functional and Bioactive Properties. *Food Chemistry*, 224, 160–171. DOI: 10.1016/j.foodchem.2016.12.057.

Walker R., Decker E.A. & McClements D.J., 2015. Development of food-grade nanoemulsions and emulsions for delivery of omega-3 fatty acids: Opportunities and obstacles in the food industry. Food & Function, 6:41–54. doi: 10.1039/C4FO00723A.

Walker, J.M. 2002. The Bicinchoninic Acid (BCA) Assay for Protein Quantitation In The Protein Protocols Handbook, 2nd Edition, Edited by: J. M. Walker © Humana Press Inc., Totowa, NJ

Wang, Y., Wang R, Chang Y, Gao Y, Li Z, Xue C. 2015. Preparation and thermo-reversible gelling properties of protein isolate from defatted Antarctic krill *Euphausia superba* byproducts. *Food Chemistry*, 188, 170–176.

Warner, R. D., 2014. Measurement of water holding capacity and colour: Objective and subjective. In *Encyclopedia of meat sciences* Elsevier, 2(2), pp. 164–171.

Waterborg, J.H. 2002. The Lowry Method for Protein Quantitation, In The Protein Protocols Handbook, 2nd Edition, Edited by: J. M. Walker © Humana Press Inc., Totowa, NJ.

Watts, M., Munday, B. L. & Burke, C. M.2001. Isolation and partial characterisation of immunoglobulin from southern bluefin tuna Thunnus maccoyii Castelnau. *Fish & Shellfish Immunology* **11,** 491-503.

Yang, S. C. & Baldwin, R. E. 2017. Functional Properties of Eggs in Foods, in Egg Science and Technology; (Stadelman, W.J.; Newkirk, D. & Newby, L.eds.) CRC Press, Boca Raton, pp 405–463.

Yousefi, N. & Abbasi, S. 2022. Food proteins: Solubility & thermal stability improvement techniques, Food Chemistry Advances, 1, 100090, https://doi.org/10.1016/j.focha.2022.100090.