Mediterranean Fisheries and Aquaculture Research

https://dergipark.org.tr/medfar

ORIGINAL RESEARCH PAPER

Received: 27 Apr. 2023 | Accepted: 22 May 2023

Biochemical and Antimicrobial Properties of *Alburnus tarichi* Roe Protein Hydrolysate

Mehmet Berköz[1*], Oruç Yunusoğlu[2], Ferbal Özkan-Yılmaz[3], Arzu Özlüer-Hunt[4], Serap Yalın[5], Ömer Türkmen[6]

[1]Department of Biochemistry, Faculty of Pharmacy, Van Yuzuncu Yıl University, Van, Turkey
[2]Department of Pharmacology, Faculty of Medicine, Bolu Abant İzzet Baysal University, Bolu, Turkey
[3]Department of Basic Sciences, Faculty of Fisheries, Mersin University, Mersin, Turkey
[4]Department of Aquaculture, Faculty of Fisheries, Mersin University, Mersin, Turkey
[5]Department of Biochemistry, Faculty of Pharmacy, Mersin University, Mersin, Turkey
[6]Department of Pharmaceutical Technology, Faculty of Pharmacy, Van Yuzuncu Yıl University, Van, Turkey

*Corresponding author e-mail: mehmet_berkoz@yahoo.com

ABSTRACT

The protein hydrolysates with antioxidant and antimicrobial potential have become a theme of great interest for pharmaceutical, nutraceuticals, as well as food processing and preservation industries. On the other hand, chemical composition of fish protein hydrolysates is significant in nutrition perspective of human health. Hence, in the present study proximate and amino acid compositions, antioxidant and antimicrobial activities of *Alburnus tarichi* roe protein hydrolysate was estimated. Protein, lipid, moisture, and ash content were found to be 87.24±0.1, 0.72±0.04, 8.79±0.3, and 7.01±0.09%, respectively. Glutamic acid, aspartic acid, lysine, leucine and alanine were found to be the most dominant amino acids, whereas, cysteine was found to be the lowest amino acid in roe protein hydrolysate. The IC_{50} value of roe protein hydrolysate for DPPH radical and hydroxyl-radical scavenging activities were 54.33 µg/mL and 77.02 µg/mL, respectively. The reducing power of *A. tarichi* roe protein hydrolysate was analyzed, the optical density values of roe protein hydrolysate and gallic acid at 700 nm were 0.45±0.03 and 1.14±0.09, respectively. Roe protein hydrolysate had shown maximum zone of inhibition against *Klebsiella pneumoniae* (11.1±0.30 mm) followed by *Salmonella enterica* (10.7±0.46 mm), *Proteus mirabilis* (10.1±0.35 mm) and *Candida albicans* (8.7±0.34 mm). The results demonstrated the importance of amino acid composition in determining the bioactive potential of the peptides. The results showed that roe protein hydrolysates of *A. tarichi* was proved to show good effect on antioxidant and antimicrobial activities and can be used a source for nutraceuticals and pharmaceuticals.

KEYWORDS: *Alburnus tarichi*, Roe protein, Protein hydrolysate, Biochemical properties, Antimicrobial activity

How to cite this article: Berköz, M., Yunusoğlu, O., Özkan-Yılmaz, F., Özlüer-Hunt, A., Yalın, S., Türkmen, Ö.(2023) Biochemical and Antimicrobial Properties of *Alburnus tarichi* Roe Protein Hydrolysate. *MedFAR*., 6(1):26-36

1. Introduction

Fish is one of the potential sources of animal protein and essential nutrients for the maintenance of a healthy body in the developing countries (Lees and Carson, 2020). Fish protein hydrolysates are reported as a source of promising health benefit components for nutritional or pharmaceutical applications (Chakniramol et al., 2022). The fish protein hydrolysates obtained after hydrolysis are composed of free amino acids and short chain of peptides showing many advantages as nutraceuticals or functional foods because of their amino acid profile. The amino acid composition of any food proteins has substantial role in numerous physiological activities of human body and affects either directly or indirectly in maintaining good health (Alboofetileh et al., 2021).

Fish protein hydrolysates have been proved to have potential role in nutritional and pharmaceutical applications (Lees and Carson, 2020; Alboofetileh et al., 2021; Chakniramol et al., 2022). Certain fish protein hydrolysates have been reported to have noticeable antioxidant activities and it has been proposed that they can be used as a natural antioxidant (Heffernan et al., 2021). The protein hydrolysates with antioxidant potential have become a theme of great interest for pharmaceutical, nutraceuticals, as well as food processing/preservation industries (Nirmal et al., 2022). On the other hand, fish exhibit different types of antimicrobial peptides. Almost all antimicrobial peptides obtained from fish have antibacterial or bacteriostatic functions against several gram-positive and gram-negative bacteria (Zamora-Sillero et al., 2018).

Alburnus tarichi, Güldenstdt, 1814 (pearl mullet) is an anadromic endemic carp species that migrates to streams (between mid-April and mid-July) living in the high pH waters of Lake Van and pouring into the lake for breeding. *A. tarichi* can leave approximately 6.000-16.000 eggs. Due to its fast growth and suitable price, *A. tarichi* currently is regarded a high economic value fish species in Turkey (Oğuz, 2018).

In this study, it was aimed to investigate the proximate composition, amino acid composition, antioxidant, and antimicrobial activities of *A. tarichi* roe protein hydrolysate.

2. Materials and Methods

2.1. Preparation of Roe Protein Hydrolysate from *A. tarichi*

A. tarichi was obtained freshly from several fishermen in Van, Turkey in March 2019. Necessary permissions were obtained from Van Animal Experiments Local Ethics Committee of Van Yuzuncu Yil University (Decision number: 2019/03, Date: 28.03.2019). Roes of the fish were isolated, homogenized, and kept at −20°C until use. Roe protein hydrolysate was prepared from defatted A. *tarichi* roe powder using Protease N (Bligh and Dyer, 1959).

2.2. Chemical Analysis of Protein Hydrolysate

2.2.1. Total Protein

The total protein content was estimated by Folin-ciocalteu method (Lowry et al., 1951).

2.2.2. Total Lipid

The lipid content was estimated according to the method of Folch et al. (1956).

2.2.3. Moisture and Ash

Moisture and ash contents were determined using the method of Kuzucu et al. (2017).

2.2.4. Amino Acid Analysis of Hydrolysate

The lyophilized hydrolysate was analyzed for the amino acid composition in the HPLC (Agilent 1100 HPLC system, Agilent Technologies, Santa Clara, CA, USA) (Baker and Han, 1994).

2.3. Antioxidant Activity

2.3.1. DPPH Radical Scavenging Assay

DPPH radical scavenging activity of hydrolysate was determined by the method followed by Wu et al. (2003).

2.3.2. Hydroxyl-radical Scavenging Assay

The hydroxyl-radical scavenging activities of the samples were analyzed by the method described by Halliwell et al. (1987).

2.3.3. Reducing Power Assay

The reducing power of the fractions of roe protein hydrolysate was measured by the method followed by Zhu et al. (2008).

2.4. Antimicrobial activity

Antimicrobial activity of roe protein hydrolysate was analyzed by well diffusion method followed by Schillinger and Luke (1989).

2.5. Statistical Analysis

All experiments were arranged in triplicate. Obtained data were presented as mean ± standard deviation. Statistical evaluation was performed using SPSS software version 15.0 (SPSS Inc., Illinois, USA). $p<0.05$ was taken into account in the evaluation of the data

3. Results

3.1. Chemical Analysis of Hydrolysate

The proximate composition of roe protein hydrolysate of *A. tarichi* was given in Table 1.

The amino acids were analyzed from the protein hydrolysate of *A. tarichi* roe. Table 2 shows the amino acid composition of protein hydrolysate of this fish. In the studied fish protein hydrolyzate, aspartic acid, leucine, lysine, alanine and glutamic acid were found the most among all the amino acids studied. Cysteine was determined as the least amino acid.

Table 1. Proximate composition (%) of roe protein hydrolysate of *A. tarichi*

Composition	Percantage (%)
Protein	87.24±0.19%
Lipid	0.72±0.04%
Moisture	8.79±0.3%
Ash	7.01±0.09%

Table 2. Amino acid composition (%) of roe protein hydrolysate of *A. tarichi*

Amino acids	Percantage (%)
Aspartic acid	10.1
Alanine	9.8
Glutamic acid	14.7
Cysteine	0.2
Proline	4.4
Glycine	6.3
Tyrosine	3.2
Arginine	5.1
Histidine	2.8
Isoleucine	3.7
Leucine	8.6
Lysine	9.9
Methionine	3.8
Phenylalanine	2.6
Threonine	5.8
Serine	4.2
Valine	4.4

3.2. Chemical Analysis of Hydrolysate

The scavenging ability in DPPH and hydroxyl-radical assays of *A. tarichi* roe protein hydrolysate in a concentration of µg/mL was found to be 63.8±2.3% and 53.8±2.8%, respectively (Figure 1 and Figure 2). The IC_{50} value of roe protein hydrolysate for DPPH radical and hydroxyl-radical scavenging activities were 54.33 µg/mL and 77.02 µg/mL, respectively.

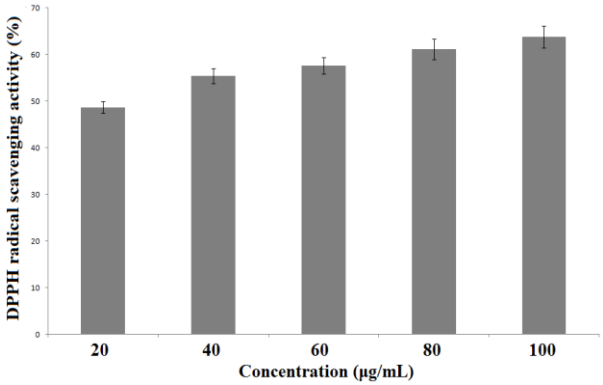

Figure 1. DPPH radical scavenging activity of roe protein hydrolysate of *A. tarichi*

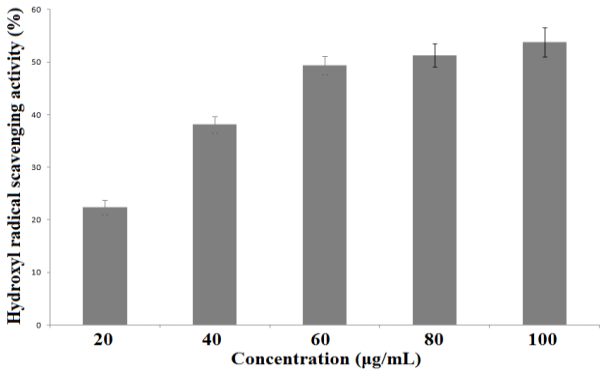

Figure 2. Hydroxyl radical scavenging activity of roe protein hydrolysate of *A. tarichi*

As a result of the analysis, the optical density values of roe protein hydrolysate and gallic acid at 700 nm were 0.45±0.03 and 1.14±0.09, respectively (Figure 3). The reducing power of gallic acid using as positive control in our study was statistically significantly higher than the protein hydrolysate ($p<0.05$).

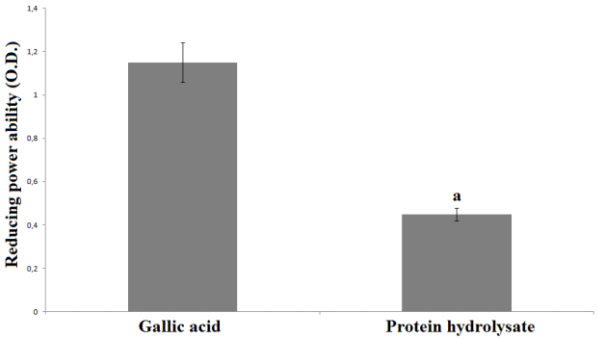

Figure 3. Hydroxyl radical scavenging activity of roe protein hydrolysate of *A. tarichi*

3.3. Antimicrobial activity

In the present study roe protein hydrolysate was analyzed for antimicrobial activity by well diffusion method. Seven human bacterial pathogens such as *E. coli*, *S. typhi*, *S. enterica*, *K. pneumoniae*, *P. mirabilis*, *S. aureus* and *V. vulnificus* and three fungal pathogens such as *C. albicans*, *C. glabrata*, and *C. tropicalis* were used for antimicrobial activity analysis. The zone of inhibition was observed against all the pathogens tested. Hence the result of roe protein hydrolysate had revealed their potent antimicrobial activity (Table 3).

Roe protein hydrolysate had shown maximum zone of inhibition against *K. pneumoniae* (11.1±0.30 mm) followed by *S. enterica* (10.7±0.46 mm), *P. mirabilis* (10.1±0.35 mm) and *C. albicans* (8.7±0.34 mm) (Table 3). Hence roe protein hydrolysate from *A. tarichi* were proved to have high antimicrobial activity against the tested human bacterial and fungal pathogens.

Table 3. Zone of inhibition produced by roe protein hydrolysate of *A. tarichi*

Pathogens	Zone of inhibition (mm)
Staphylococcus aureus	7.1±0.24
Vibrio vulnificus	8.1±0.46
Proteus mirabilis	10.1±0.35
Klebsiella pneumoniae	11.1±0.30
Salmonella enterica	10.7±0.46
Salmonella typhi	5.6±0.23
Escherichia coli	7.6±0.39
Candida albicans	8.7±0.34
Candida glabrata	7.6±0.28
Candida tropicalis	7.1±0.56

4. Discussion and Conclusion

The chemical content of fish protein hydrolysates is important for a healthy diet of humans. (Lees and Carson, 2020; Alboofetileh et al., 2021; Chakniramol et al., 2022; Borges et al., 2023; Langlois et al., 2023). In the present study the protein content of roe protein hydrolysate of *A. tarichi* was 87.24%. Hence, this protein hydrolysate was rich in protein content, it could be considered as an essential source of proteins. Similar result was recorded by Kristinsson and Rasco (2000) and Pacheco-Aguilar et al. (2008) in *Salmo salar* (88.39%) and *Merluccius productus* (88.6%) muscle protein hydrolysates, respectively. The protein content of fish muscle hydrolysates has been reported as 60-97% of the total composition. (Kristinsson and Rasco, 2000). The excess content indicated for fish protein hydrolysates can be explained by the dissolution of the proteins during hydrolysis and also the removal of insoluble solid particles by centrifugation (Chala-maiah et al., 2012). The high protein content of the investigated fish hydrolysates made it suitable as a dietary supplement.

We found the lipid content of roe protein hydrolysate of *A. tarichi* as 0.72%, similar result was reported from *Lutjanus vitta* muscle protein hydrolysates (0.64%) by Khantaphant and Benjakul (2008) and *Oreochromis niloticus* meat hydrolysates (0.67%) by Foh et al. (2011). The lipid content of most of the fish muscle protein hydrolysates were reported as less than 5% by several researchers in various fish species (Kristinsson and Rasco, 2000, Pacheco-Aguilar et al., 2008, Khantaphant and Benjakul, 2008). It accounts for the low oil content of fish protein hydrolysates due to the elimination of undissolved compounds and lipids by centrifugation (Shahidi et al., 1995).

Moisture content was 8.79% in the current study, almost similar value of moisture content was reported by Thiansilakul et al. (2007) and Dong et al. (2005) in *Decapterus maruadsi* (8.75%) and *Saurida elongata* (8.5%) protein hydrolysates. But the present value was lower than the value reported by Khantaphant and Benjakul (2008) in *Lutjanus vitta* muscle protein hydrolysates (11.16%). It causes low moisture content in protein hydrolysates due to the type of samples and oven drying and evaporation processes (Bueno-Solano et al., 2009). Most studies have proven that fish protein hydrolysates from various species contain less than 10% moisture (Kristinsson and Rasco, 2000, Pacheco-Aguilar et al., 2008, Choi et al., 2009).

In the present study, the ash content was 7.01%. Dong et al. (2005) recorded almost similar value of ash in *Saurida elongata* protein hydrolysates (7.1%). The ash content of most of the fish muscle protein hydrolysates was reported as 1.76% to 24.56% of total composition (Kristinsson and Rasco, 2000, Thiansilakul et al., 2007, Pacheco-Aguilar et

al., 2008, Choi et al., 2009, Foh et al., 2011). The ash content of fish protein hydrolysates may have been found to be high due to the acid or base substances added to balance the pH of the medium (Kristinsson and Rasco, 2000, Dong et al., 2005, Pacheco-Aguilar et al., 2008, Choi et al., 2009).

Among the amino acids studied aspartic and glutamic acid and lysine were found to be dominant in the protein hydrolysate prepared from *A. tarichi* roe. This result shows good agreement with the amino acid composition of various fish protein hydrolysates (Klompong et al., 2009, You et al., 2011, Ghassem et al., 2014). In this present study, amount of glutamic acid was recorded in *A. tarichi* roe protein hydrolysate to be 16.5%, almost similar value was recorded by Ghassem et al. (2014) in *Channa striatus* myofibrillar protein hydrolysates (16.4%) produced by proteinase K and You et al. (2011) in *Misgurnus anguilliacaudatus* protein hydrolysates (16.7%). The amount of aspartic acid was recorded to be 10.1%. Similarly, Khantaphant et al. (2008) recorded 10.65% and 10.73% of aspartic acid in *Lutjanus vitta* muscle protein hydrolysates. Lysine was also found to be dominant in *A. tarichi* to be 10.1%, almost similar value was recorded by Ghassem et al. (2014) in *Channa striatus* sarcoplasmic protein hydrolysates (9.9 ± 0.64%) produced by proteinase K. Fish protein hydrolysates have been reported to exhibit differences in their amino acid compositions (You et al., 2011, Hou et al., 2011, Ghassem et al., 2014). Some factors such as hydrolysis conditions, type of enzyme, raw material may be the reason for the difference in amino acid composition of fish protein hydrolysates (Klompong et al., 2009).

Protein hydrolysates of various fish sources such as whole capelin, tuna cooking juice, skin gelatin from brown stripe red snapper, mackerel, yellow fin sole frame, Alaska pollack frame, round scad muscle, Atlantic salmon, hoki, conger eel, yellow travelly, mackerel, loach and Pacific hake muscle have been proved to have antioxidant activity (Klompong et al., 2009). Some studies have shown that there is a good correlation between certain amino acid residues and the antioxidant potential of protein hydrolysates (González-Serrano et al., 2022). It is accepted that peptides with higher content of lysine, tyrosine, histidine, methionine, tryptophan, proline, alanine, valine and leucine have more antioxidant properties (Liu et al., 2021). The protein hydrolysates prepared from *A. tarichi* roe contained all these antioxidants related amino acids. Hence the present study has also focused on the antioxidant activity of protein hydrolysate prepared from *A. tarichi* roe. For this aim, DPPH and hydroxyl radical scavenging assays were used for finding out the antioxidant activity of the protein hydrolysate of *A. tarichi* roe.

DPPH, one of the organic nitrogen radicals, takes an electron or hydrogen atom, becoming a stable and commercially stable diamagnetic molecule (Mäkinen et al., 2022). Maximum DPPH radical scavenging activity of the protein hydrolysate of *A. tarichi* was found to be 63.8%. Sampath Kumar et al. (2011) reported almost similar value of antioxidant activity in the crude fish protein hydrolysate prepared from the horse mackerel, *Magalaspis cordyla* viscera protein to be 61.1%. Similarly, Nazeer et al. (2012) recorded to be 59.7% of DPPH radical scavenging activity in croaker muscle protein hydrolysates. The hydroxyl radical is an extremely reactive free radical that can severely damage polymer biomolecules such as DNA, fatty acids, proteins (Zhou et al., 2012). Maximum hydroxyl radical scavenging activity of the protein hydrolysate of *A. tarichi* was found to be 53.8%. Almost similar value was recorded by Sampath Kumar et al. (2011) in the horse mackerel visceral protein hydrolysates (48.3%) and Nazeer et al. (2012) in the croaker muscle protein hydrolysates (49.7%). In the present study the IC_{50} value of protein hydrolysates of *A. tarichi* roe for DPPH and hydroxyl radical scavenging activities were recorded as 54.33 μg/mL and 77.02 μg/mL, respectively. Similarly, Ranathunga et al. (2006) reported IC_{50} value of 74.1 μM for hydroxyl radical scavenging activity of antioxidative peptide derived

from muscle of conger eel (*Conger myriaster*). However, Kim et al. (2007) reported IC_{50} value of hoki frame protein hydrolysates for DPPH and hydroxyl as 41.37 μM and 17.77 μM respectively. Sun et al. (2013) also reported that IC_{50} value of tilapia gelatin peptides for hydroxyl radical scavenging activity was 22.47 μg/mL. The DPPH radical scavenging property may support that hydrolysate have hydrophobic amino acid content, which is more suitable for oil-soluble radicals of DPPH. The hydroxyl radical can be scavenged by the electron/hydrogen donor process by protein hydrolysates. The activity of the hydroxyl radical is the strongest among the free oxygen species, since it reacts more easily with biomolecules such as protein, lipid, and DNA. Since it reacts more easily with biomolecules such as protein, lipid and DNA, the activity of the hydroxyl radical is the strongest among the free oxygen species. Therefore, neutralization of the hydroxyl radical is perhaps the most effective defense for a living cell or organ (Liu et al., 2023).

The reducing power of roe protein hydrolysate exhibited the optical density value of 0.45±0.03. Free radicals are reduced by electron donating. The electron donating probability indicates the reducing ability of a compound, which is its antioxidant property (Zhang et al., 2023). The reduction potential of protein hydrolysates has been determined, but the details are not clear. The reductants can reduce free radicals by transmitting electrons to them. The electron donating possibility of a given compound, termed as reducing ability, may serve as a substantial indicator of its probable antioxidant activity (Zhang et al., 2023). The reducing power ability is observed commonly from protein hydrolysates, but the exact nature is still unclear.

In some studies, it has been revealed that the peptides of the fish proteins obtained have stronger antioxidant potential in different oxidative systems (Sun et al., 2021). Although the information about the antioxidant function of peptides is not known in detail, it has been stated that histidine and a few aromatic amino acids are of great importance in this case. Antioxidant activity of protein hydrolysates mainly depends on the peptides present in the hydrolysates. The hydrolysates, which are rich in peptides containing especially hydrophobic amino acids are supposed to possess high antioxidant activity (Heffernan et al., 2021). As a result, we can suggest that the amino acid composition of peptides in protein hydrolysates is a critical factor in directing the antioxidant activity of protein hydrolysates.

In antimicrobial activity roe protein hydrolysate tested against seven bacterial pathogens and three fungal pathogens. Roe protein hydrolysate of *A. tarichi* was proved to have antimicrobial activity against the tested human bacterial and fungal pathogens. This result was supported by Ramkumar et al. (2005), who reported antibacterial activity of epidermal layer of coral reef associated fish of Tuticorin coast and proved that antibacterial activity of *Siganus canaliculatus* against eight bacterial pathogens. Wang et al. (2011) also reported that the serum sample from rabbit fish (*Siganus oramin*) can inhibit the growth of both gram-negative bacteria and gram-positive bacteria especially *B. subtilis*, *E. coli* and *A. sobria* were highly sensitive to the serum.

Furthermore, the protein hydrolysates obtained from various fish sources were proved to have antimicrobial activity. Song et al. (2012) proved that pepsin hydrolysate of half-fin anchovy (*Setipinna taty*) have antibacterial activity against *E. coli*. Sila et al. (2014), reported that peptides from the protein hydrolysates of *Barbus callensis* muscle showing antibacterial activity against the gram-positive (*S. aureus, Listeria monocytogenes*, *Micrococcus luteus*, *Enterococcus faecalis* and *Bacillus cereus*) and the gram-negative (*Pseudomonas aeruginosa*, *E. coli*, *K. pneumoniae*, *S. enterica* and *Enterobacter sp*.) bacterias. Tang et al. (2015), stated that Anchovy (*Engraulis japonicus*) cooking wastewater protein hydrolysates having antimicrobial activity. And also, the second structure of peptides, peptide content, amino acid sequence are important factors that

change the strength of antibacterial activity (Shahidi and Zhong, 2008).

In conclusion, the results demonstrated the importance of amino acid composition in determining the bioactive potential of the peptides. The results showed that roe protein hydrolysates of *A. tarichi* was proved to show good effect on antioxidant and antimicrobial activities and can be used a source for nutraceuticals and pharmaceuticals, indeed in vivo studies are needed.

Compliance with Ethical Standards

This study was presented as a poster presentation at the 13th International Symposium on Pharmaceutical Sciences (ISOPS-2021) (Poster No: 178).

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Ethical approval

For this study, necessary permissions were obtained from Van Animal Experiments Local Ethics Committee of Van Yuzuncu Yil University (Decision number: 2019/03, Date: 28.03.2019).

References

Alboofetileh, M., Hamzeh, A., Abdollahi, M. (2021) Seaweed proteins as a source of bioactive peptides. Current Pharmaceutical Design 27: 1342–1352.

Baker, D.H., Han, Y. (1994) Ideal amino acid profile for broiler chicks during three weeks post hatching. Poultry Science 73: 1441-1447.

Bligh, E.G., Dyer, W.J. (1959) A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology 37: 911-917.

Borges, S., Odila, J., Voss, G., Martins, R., Rosa, A., Couto, J.A., Almeida, A., Pintado, M. (2023) Fish by-products: A source of enzymes to generate circular bioactive hydrolysates. Molecules (Basel) 28: 1155.

Bueno-Solano, C., Lopez-Cervantes, J., Campas-Baypoli, O. N., Lauterio-Garcia, R., Adan-Bante, N.P., Sanchez-Machado, D.I. (2009) Chemical and biological characteristics of protein hydrolysates from fermented shrimp by-products Food Chemistry 112: 671-675.

Chakniramol, S., Wierschem, A., Cho, M.G., Bashir, K.M.I. (2022) Physiological and clinical aspects of bioactive peptides from marine animals. Antioxidants (Basel) 11: 1021.

Chalamaiah, M., Dinesh-Kumar, B., Hemalatha, R., Jyothirmayi, T. (2012) Fish protein hydrolysates: proximate composition, amino acid composition, antioxidant activities and applications: a review. Food Chemistry 135: 3020-3038.

Choi, Y.J., Hur, S., Choi, B.D., Konno, K., Park, J.W. (2009) Enzymatic hydrolysis of recovered protein from frozen small croaker and functional properties of its hydrolysates. Journal of Food Science 74: 17-24.

Dong, Y., Sheng, G., Fu, J., Wen, K. (2005) Chemical characterization and anti-anaemia activity of fish protein hydrolysate from Saurida elongate. Journal of the Science of Food and Agriculture 85: 2033-2039.

Foh, M.B.K., Kamara, M.T., Amadou, I., Foh, B.M., Wenshui, X. (2011) Chemical and physiochemical properties of Tilapia (Oreochromis niloticus) fish protein hydrolysates and concentrate. International Journal of Biological Chemistry 5: 21-36.

Folch, J., Lees, M., Sloane-Stanley, G.H. (1956) A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry 226: 497-509.

Ghassem, M., Fern, S.S., Said, M., Ali, Z.M., Ibrahim, S., Babji, A.S. (2014) Kinetic characterization of Channa striatus muscle sarcoplasmic and myofibrillar protein hydrolysates. Journal of Food Science and Technology 51: 467-475.

González-Serrano, D.J., Hadidi, M., Varcheh, M., Jelyani, A.Z., Moreno, A., Lorenzo, J.M. (2022) Bioactive peptide fractions from collagen hydrolysate of common carp fish byproduct: Antioxidant and functional properties. Antioxidants (Basel) 11: 509.

Halliwell, B., Gutteridge, J.M.C., Aruoma, O.I. (1987) The deoxyribose method: A simple "test-tube" assay for determination of rate contents for reactions of hydroxyl radicals. Analytical Biochemistry 165: 215-219.

Heffernan, S., Giblin, L., O'Brien, N. (2021) Assessment of the biological activity of fish muscle protein hydrolysates using in vitro model systems. Food Chemistry 359: 129852.

Hou, H., Li, B., Zhao, X. (2011) Enzymatic hydrolysis of defatted mackerel protein with low bitter taste. Journal of Ocean University of China 10: 85-92.

Khantaphant, S., Benjakul, S. (2008) Comparative study on the proteases from fish pyloric caeca and the use for production of gelatin hydrolysate with anti- oxidative activity. Comparative Biochemistry and Physiology Part B 151: 410-419.

Kim, S., Je, J., Kim, S. (2007) Purification and characterization of antioxidant peptide from hoki (Johnius belengerii) frame protein by gastrointestinal digestion. The Journal of Nutritional Biochemistry 18: 31-38.

Klompong, V., Benjakul, S., Yachai, M., Visessanguan, W., Shahidi, F., Hayes, K.D. (2009) Amino acid composition and antioxidative peptides from protein hydrolysates of yellow stripe trevally (Selaroides leptolepis). Journal of Food Science 74: 126-133.

Kristinsson, H.G., Rasco, B.A. (2000) Fish protein hydrolysates: production, biochemical, and functional properties. Critical Reviews in Food Science and Nutrition 40: 43-81.

Kuzucu, M. (2017) Effects of water harvesting techniques and using humic acid on soil moisture, plant evaporation, growth and yield in pistachio orchards in southeastern of Turkey. Fresenius Environmental Bulletin 26: 7521-7528.

Langlois, L.D., Oddoux, S., Aublé, K., Violette, P., Déchelotte, P., Noël, A., Coëffier, M. (2023) Effects of glutamine, curcumin and fish bioactive peptides alone or in combination on intestinal permeability in a chronic-restraint stress model. International Journal of Molecular Sciences 24: 7220.

Lees, M.J., Carson, B.P. (2020) The potential role of fish-derived protein hydrolysates on metabolic health, skeletal muscle mass and function in ageing. Nutrients 12: 2434.

Liu, C., Chen, G., Rao, H., Xiao, X., Chen, Y., Wu, C., Bian, F., He, H. (2023) Novel antioxidant peptides identified from Arthrospira platensis hydrolysates prepared by a marine Bacterium pseudoalteromonas sp. JS4-1 extracellular protease. Marine Drugs 21: 133.

Liu, W.Y., Zhang, J.T., Miyakawa, T., Li, G.M., Gu, R.Z., Tanokura, M. (2021) Antioxidant properties and inhibition of angiotensin-converting enzyme by highly active peptides from wheat gluten. Scientific reports 11: 5206.

Lowry, O.H., Rosebrough, N.J., Farr, A.C. (1951) Protein measurement with the foli-phenol reagent. Journal of Biological Chemistry 193: 265-275.

Mäkinen, S., Hiidenhovi, J., Huang, X., Lima, A.D.S., Azevedo, L., Setälä, J., Välimaa, A.L., Mattila, P., Granato, D. (2022) Production of bioactive peptides from Baltic herring (Clupea harengus membras): Dipeptidyl peptidase-4 inhibitory, antioxidant and antiproliferative properties. Molecules (Basel) 27: 5816.

Nazeer, R.A., Sampath-Kumar, N.S., Jai-Ganesh, R. (2012) In vitro and in vivo studies on the antioxidant activity of fish peptide isolated from the croaker (Otolithes ruber) muscle protein hydrolysate. Peptides 35: 261-268.

Nirmal, N.P., Santivarangkna, C., Rajput, M.S., Benjakul, S., Maqsood, S. (2022) Valorization of fish byproducts: Sources to end-product applications of bioactive protein hydrolysate. Comprehensive Reviews in Food Science and Food Safety 21: 1803-1842.

Oğuz, A.R. (2018) Development of osmoregulatory tissues in the Lake van fish (*Alburnus tarichi*) during larval development. Fish Physiology and Biochemistry 44: 227-233.

Pacheco-Aguilar, R., Mazorra-Manzano, M.A., Ramirez-Suarez, J.C. (2008) Functional properties of fish protein hydrolysates from Pacific whiting (*Merluccius productus*) muscle produced by a commercial protease. Food Chemistry 109: 782-789.

Ram-Kumar, R., Senthil-Kumar, S., Patterson-Edward, J.K. (2005) Antibacterial activity of the epidermal layer of coral associated fishes of Tuticorin coast. Indian Journal of Fisheries 52: 119-124.

Ranathunga, S., Rajapakse, N., Kim, S.K. (2006) Purification and characterization of antioxidative peptide derived from muscle of conger eel (Conger myriaster). European Food Research and Technology 22: 310-315.

Sampath-Kumar, N.S., Nazeer, R.A., Jaiganesh, R. (2011) Purification and identification of antioxidant peptides from the skin protein hydrolysate of two marine fishes, horse mackerel (Magalaspis cordyla) and croaker (Otolithes ruber). Amino Acids 42: 1641-1649.

Schillinger, V., Luke, K.K. (1989) Antibacterial activity of Lactobacillus sake isolated from meat. Applied and Environmental Microbiology 55: 1091-1096.

Shahidi, F., Han, X., Synowiecki, J. (1995) Production and characteristics of protein hydrolysates from capelin (Mallotus villosus). Food Chemistry 53: 285-293.

Shahidi, F., Zhong, Y. (2008) Bioactive peptides. Journal of AOAC International 91: 914-931.

Sila, A., Nedjar-Arroume, N., Hedhili, K., Chataigne, G., Balti, R., Nasri, M., Bougatef, A. (2014) Antibacterial peptides from barbel muscle protein hydrolysates: Activity against some pathogenic bacteria. LWT - Food Science and Technology 55: 183-188.

Song, R., Wei, R.B., Luo, H.Y., Wang, D.F. (2012) Isolation and characterization of an antibacterial peptide fraction from the pepsin hydrolysate of half-fin anchovy (Setipinna taty). Molecules 17: 2980-2991.

Sun, L., Zhang, Y., Zhuang, Y. (2013) Antiphotoaging effect and purification of an antioxidant peptide from tilapia (Oreochromis niloticus) gelatin peptides. Journal of Functional Foods 5: 154-162.

Sun, X., Wang, K., Gao, S., Hong, H., Zhang, L., Liu, H., Feng, L., Luo, Y. (2021) Purification and characterization of antioxidant peptides from yak (Bos grunniens) bone hydrolysates and evaluation of cellular antioxidant activity. Journal of Food Science and Technology 58: 3106-3119.

Tang, W., Zhang, H., Wang, L., Qian, H., Qi, X. (2015) Targeted separation of antibacterial peptide from protein hydrolysate of anchovy cooking wastewater by equilibrium dialysis. Food Chemistry 168: 115-123.

Thiansilakul, Y., Benjakul, S., Shahidi, F. (2007) Compositions, functional properties and antioxidative activity of protein hydrolysates prepared from round scad (Decapterus maruadsi). Food Chemistry 103: 1385-1394.

Wang, F.H, Li, R.J., Xie, M.Q., Li, A.X. (2011) The serum of rabbitfish (Siganus oramin) has antimicrobial activity to some pathogenic organisms and a novel serum L amino acid oxidase is isolated. Fish & Shellfish Immunology 30: 1095-1108.

Wu, H.C., Chen, H.M., Shiau, C.Y. (2003) Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (*Scomber austriasicus*). Food Research International 36: 949-957.

You, L., Zhao, M., Regenstein, J.M., Ren, J. (2011) In vitro antioxidant activity and in vivo anti-fatigue effect of loach (Misgurnus anguillicaudatus) peptides prepared by papain digestion. Food Chemistry 124: 188-194.

Zamora-Sillero, J., Gharsallaoui, A., Prentice, C. (2018) Peptides from fish by-product protein hydrolysates and its functional properties: an overview. Marine Biotechnology 20: 118-130.

Zhang, H., Yang, X., Zhong, R., Huo, Y., Zhu, Y., Liang, P. (2023) Antioxidative properties of fish roe peptides combined with polyphenol on the fish oil oleogel. Journal of the Science of Food and Agriculture 103: 1714-1726.

Zhou, D., Tang, Y., Zhu, B., Lei, Q., Li, D., Yang, J., Lei, K. (2012) Antioxidant activity of hydrolysates obtained from scallop (Patinopecten yessoensis) and abalone (Haliotis discus hannai Ino) muscle. Food Chemistry 132: 815-822.

Zhu, B.W., Wang, L.S., Zhou, D.Y., Li, D.M., Sun, L.M., Yang, J.F., Wu, H.T., Zhou, X.Q., Tada, M. (2008) Antioxidant activity of sulphated polysaccharide conjugates from abalone (Haliotis discus hannai Ino). European Food Research and Technology 227: 663-668.