ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2022; 27(2): 3362-3367
doi: 10.25083/rbl/27.2/3362.3367

Received for publication: December, 08, 2021
Accepted: March, 17, 2022

Original paper

Enzymatic extraction, characterization and biological properties of protein hydrolysates from freshwater fish waste

TOMA AGNES[1,2], CRACIUNESCU OANA[2], MOLDOVAN LUCIA[2], CIUCAN TEODORA[2], TATIA RODICA[2], ILIE DANIELA[2], MIHAI ELENA[2], SAVIN SIMONA[2], SANDA CATALINA[2], ANCA OANCEA[2], JURCOANE STEFANA[1], ISRAEL FLORENTINA[1], BALAN DANIELA[1], LUTA GABRIELA[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]National Institute of Research and Development for Biological Sciences, 296, Splaiul Independentei, Bucharest, Romania

Abstract

Silver carp *(H. molitrix*) is one of the most popular species in fish farms around the world. In this paper, the bioactive properties of four protein hydrolysates from silver carp residues obtained with papain, flavourzyme, alcalase and combination of flavourzyme & alcalase treatment were analyzed. Physicochemical characterization showed that the alcalase extract had the highest extraction yield of 52.07%, presented 90.33% protein content and the highest degree of hydrolysis (76.23%). Gel electrophoresis pattern indicated that hydrolysate obtained with alcalase contains most peptides with a molecular weight below 15 kDa, while those present in the hydrolysate obtained with flavourzyme & alcalase had a molecular weight between 10-15 kDa. The effect of protein hydrolysates on DPPH free radicals inhibition varied between 47.37-50.14%, the highest value of antioxidant activity being recorded for hydrolysate obtained with papain. The hydrolysates presented antihypertensive potential determined as inhibition of angiotensin-converting enzyme, with the highest activity in the case of flavourzyme & alcalase extract. The fish protein hydrolysates were cytocompatible in a normal fibroblast culture, NCTC cell line showing cell viability over 80% for all variants. When cultivated in Hep-2 cancer cells at 10 mg/ml, the protein hydrolysates obtained by papain and flavourzyme & alcalase mixture decreased the cell viability, indication antitumoral potential. In conclusion, the fish protein hydrolysates demonstrated important bioactive properties, including antioxidant, antihypertensive and antiproliferative activity.

Keywords bioactive peptides, fish hydrolysate, antioxidant, antihypertensive, antitumoral

To cite this article: TOMA A, CRACIUNESCU O, MOLDOVAN L, CIUCAN T, TATIA R, ILIE D, MIHAI E, SAVIN S, SANDA C, OANCEA A, JURCOANE S, ISRAEL F, BALAN D, LUTA G. Enzymatic extraction, characterization and biological properties of protein hydrolysates from freshwater fish waste. *Rom Biotechnol Lett.* 2022; 27(2): 3362-3367 DOI: 10.25083/rbl/27.2/3362.3367

*Corresponding author: Agnes Toma, National Institute of Research and Development for Biological Sciences 296, Splaiul Independentei, 060031 Bucharest, Romania, Tel: +40-21-2200882, E-mail: agnes.toma@incdsb.ro

Introduction

Fish processing industry generates huge amount of wastes (skin, scales, bones, internal organs) that could be valorized to extract useful compounds, such as collagen, gelatin, bioactive peptides and minerals using physicochemical and enzymatic methods.

Fish protein hydrolysis can be performed by chemical (acidic, basic) or enzymatic treatment. The latter is easier to control, because enzymes cut proteins at certain sites resulting in certain peptides and the best results are obtained under specific conditions (pH, temperature, exposure time). The enzymes used for this purpose can have microbial (flavourzyme, alcalase, neutrase, protamex), plant (bromelain, ficin, papain) and animal (trypsin, pepsin, chymotrypsin) origin (Gao, 2021). Protein hydrolysates, resulting from the hydrolysis of native proteins, contain polypeptides and small bioactive peptides of 2-20 amino acids (Chalamaiah, 2012). Previous studies have showed that fish hydrolysates present antioxidant properties both *in vitro* and *in vivo*, antitumor, anti-inflammatory, antihypertensive, neuroprotective and antibacterial properties (Gao, 2021). The beneficial properties of the hydrolysates depend on the hydrolysis degree of the peptide extracts, composition and size of the constituent peptides.

Silver carp is a freshwater fish of the cyprinid family found in farms around the world. The by-products resulting from its processing could be used to obtain protein hydrolysates and bioactive peptides by enzymatic hydrolysis. It was previously showed that enzyme hydrolysates of silver carp white muscle obtained by alcalase, flavourzyme, neutrase, papain, pepsin, protamex and trypsin treatment presented antioxidant activity with the highest value in the case of pepsin hydrolysates (Zhong et al., 2011). Analyzing silver crap fins hydrolysates obtained with papain, alcalase, neutrase or trypsin, the highest antioxidant activity was reported in the case of alcalase and trypsin treatment (Zhang et al., 2020). Wang studied the enzymatic hydrolysates of silver carp muscle in terms of antioxidant properties assessed as free radicals inhibition and their biological properties in Caco2 cell cultures (Wang et al., 2021).

The present paper aimed to obtain enzymatic protein hydrolysates from silver carp waste tissues (bones, meat, skin) using the following types of enzymes: papain, flavourzyme, alcalase, and a combined treatment with flavourzyme & alcalase. The physicochemical characterization of the four hydrolysates was performed in terms of extraction yield, protein content, degree of hydrolysis and gel electrophoresis, then their antioxidant and antihypertensive activity assessment, while the biological evaluation consisted of biocompatibility and antiproliferative activity testing using stabilized cell lines.

Materials and methods

Obtaining fish protein hydrolysates

Fish waste tissues were pretreated by washing, decalcification and delipidization and then subjected to hydrolysis with papain in phosphate buffer 7.5µg/ml at pH 6, flavourzyme in phosphate buffer 4.5 µg/ml at pH 7, alcalase in phosphate buffer 4.5µg/ml at pH 8 and a combined treatment with flavourzyme at pH 7 followed by alcalase at pH 8 (Zamora-Sillero, 2018).

Yield and protein concentration

The extraction yield was calculated as percentage reported to the amount of initial tissue put into work, based on the dry weight, according to the following formula.

Extraction yield (%) = final dry weight product / initial dry weight of raw material x 100

Protein quantification was done using Biuret assay based on reaction of $CuSO_4$ with peptide bonds resulting in a purple colored complex. The absorbance was read at 540 nm at a Spectrostar nano microplate reader (BMG Labtech). A standard curve was built using bovine serum albumin in a range of 0-200 µg/ml to calculate the protein concentration (Zheng et al., 2017)

Determination of the degree of hydrolysis using TNBS assay

The TNBS assay was used to quantify the free amino groups in the protein hydrolysate samples (Adler-Nissen 1969). Trinitrobenzenesulfonic acid (TNBS) reagent was prepared in 0.05 M Tris buffer, pH 8.3. A solution of L-leucine was used as standard. Absorbance was read at 346 nm at a V-650 UV-VIS spectrophotometer (Jasco, Japan).

Gel electrophoresis

Samples of protein hydrolysates were migrated in Tricine-SDS-polyacrylamide gel in 10-20% gradient of concentration, alongside a molecular weight marker. Before migration, the samples were mixed with Tricine SDS sample buffer and a reducing agent. After migration, the proteins were fixed in the gel by incubation in 85% o-phosphoric acid solution containing methanol, for 60 min. Then, the gel was stained by incubation in Coomassie blue solution, for 12 h and destained in 25% methanolic solution (Schagger, 2006).

Antioxidant activity assay

The antioxidant activity was analyzed using DPPH method (Zhang et al., 2011). This method is based on reducing

the DPPH radical in solution, which has red-purple color, turning to yellow colored solution. The absorbance was read at 520 nm using a V-650 UV-VIS spectrophotometer (Jasco, Japan). The degree of inhibition of the DPPH radical was calculated using the formula:

%DPPH inhibition=(1–sample absorbance/blank absorbance)×100

Determination of angiotensin-converting enzyme (ACE) inhibition

To determine the inhibitory potential of protein hydrolysates on the ACE, the samples were mixed with the substrate hippuryl-L-histidyl-L-leucine solution, dissolved in sodium borate buffer and NaCl, and then, with ACE. The absorbance was measured at 228 nm. The inhibition of the enzyme activity was expressed as percentage from initial activity (Papadimitriou et al 2012).

Cell cytotoxicity tests

The cytotoxicity evaluation of peptides samples was performed according to the standard SR-EN IS 10993. The samples were tested i*n vitro* on normal mouse fibroblast NCTC cells and human tumor epithelial Hep-2 cells, using MTT cell viability assay.After 48 hours of treatment, the samples were treated with tetrazolium bromide salt solution (MTT) and isopropanol. The absorbance of resulted solutions was measured at the wavelength of 570 nm, using a Berthold plates reader (Germany). Cell viability was determined with the formula: %cell viability=(sample O.D./control O.D.)×100, where the viability of the Control culture is considered to be 100%.

Results and discussion

Yield and protein content

The extraction yield and protein content of the four enzymatic hydrolysates varied between 20.94-52.07% and 32.32-95%, respectively (Table 1). The lowest extraction yield was recorded in the case of papain hydrolysis (20.94%), but the extract had the highest protein content of 95%. These values were higher than those obtained by Noman et al. (2018) which reported values of 17.47% for yield and 79.67% for protein content as results of Chinese stugeron hydrolysis with papain. The hydrolysate obtained by alcalase treatment had the highest extraction yield (52.07%), which was 248.66% higher than the papain hydrolysate yield, while the protein content was 90.33%. In the case of hydrolysates prepared by flavourzyme and flavourzyme & alcalase treatment, the extraction yields were 48.4% and 50.4%, respectively, while the protein content was 32.32% and 58.11%, respectively.

Degree of hydrolysis

The degree of hydrolysis performed with different enzymatic treatments influences the peptides size and their biological properties. In our study, the degree of hydrolysis after treatment with papain, flavourzyme and alcalase varied between 61.4 -76.23% (Table 1). The highest degree of hydrolysis was obtained in the case of alcalase treatment. Similar results regarding the degree of hydrolysis of fish proteins after alcalase and papain treatment were reported by Je et al. (2007) in a study analyzing the hydrolysates from tuna backbone. Other researches noted higher hydrolysis degree of peptide extracts from fish by-products obtained with alcalase, compared to that of extracts obtained with other enzymes (papain, neutrase, flavourenzyme, protamex) (Idow et al., 2018; Zhang et al. al., 2021).

Gel electrophoresis

The pattern of protein hydrolysates migration in 10-20% gradient gels of SDS-tricine-polyacrylamide gel allowed the evaluation of their molecular weight. Following electrophoresis, polypeptides with a molecular weight between 10-15 kDa were observed in high proportion in all analyzed hydrolysates (Fig. 1). The hydrolysate obtained by flavourzyme treatment also presented peptides with molecular weight around 40 kDa. Instead, the hydrolyzate obtained by alcalase treatment contained mainly peptides with low molecular weight between 3-12 kDa. Previous studies of Roslan et al. (2014) reported the presence of low molecular weight peptides (below 3.5 kDa) in the protein hydrolysate obtained from tilapia by-products using alcalase, as showed the SDS-tricine-polyacrylamide electrophoresis analysis.

Antioxidant activity

In our study, the enzymatic hydrolysis of fish by-products resulted in bioactive peptides able to neutralize free radicals by donating an electron or a proton. Determination of the antioxidant activity by DPPH assay in the obtained hydrolysates showed that the values of DPPH radicals inhibition

Table 1. Extraction yield, protein content and hydrolysis degree of protein hydrolysates. The results are expressed as mean ± SD (n=3).

	Papain hydrolysate	Flavourzyme hydrolysate	Alcalase hydrolysate	Flavourzyme & alcalase hydrolysate
Extraction yield [%]	20.94 ±2.52	48.45 ±2.77	52.07 ±2.31	50.40±2.64
Protein content [%]	95.00 ±0.035	32.32 ±0.02	90.33 ±0.013	58.11 ±0.021
Hydrolysis degree [%]	61.40 ±0.26	66.33 ±0.1	76.23 ±0.15	67.50±0.23

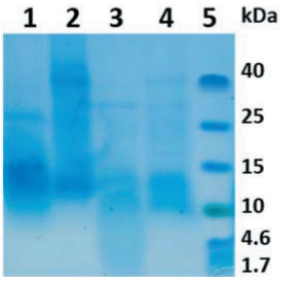

Figure 1. Gel electrophoresis of protein hydrolysates obtained by papain (1), flavourzyme (2), alcalase (3) and flavourzyme & alcalase (4) treatment. A low molecular weight marker (1.7-40 kDa) (5) was migrated in the same gel.

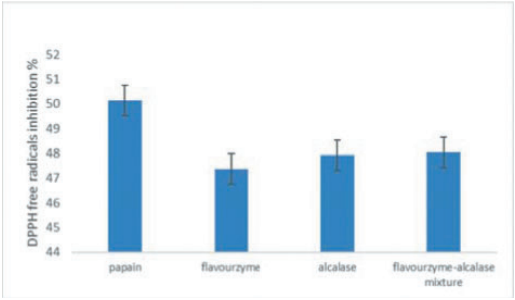

Figure 2. The antioxidant activity of the enzymatic hydrolysates determined as DPPH free radicals inhibition. The results are expressed as mean ± SD (n=3).

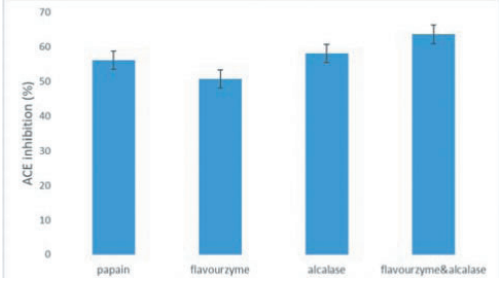

Figure 3. Inhibition potential of protein hydrolysates on ACE. The results are expressed as mean ± SD (n=3).

degree varied in a narrow range between 47.37-50.14%, at a concentration of 10 mg/ml enzyme fish hydrolysate (Fig. 2).

Numerous studies have demonstrated the antioxidant effects of different fish hydrolysates obtained with different enzymatic Peptides isolated from bones, meat, viscera and skin with alcalase, chymotrypsin, papain, pepsin, flavourzyme, protamex, neutrase have been shown to have antioxidant activity (Chalamaiah et al., 2012; Idow et al., 2018; Tacias-Pascacio et al., 2021). Je et al. (2007) analyzed the degree of inhibition of several hydrolysates obtained by treatment of tuna backbone with different enzymes and reported the highest value in the case of papain (36.72%) and the lowest in the case of alcalase (4.82%). Other studies analyzing the antioxidant activity of enzymatic hydrolysates from Alaska pollock skin by DPPH assay showed similar values of inhibition degree (32-50%) and the highest antioxidant activity for peptides extract obtained by flavourzyme treatment (Jia et al., 2010). Li et al (2012) reported that higher DPPH inhibition degree was noted in the case of hydrolyzate of grass carp meat obtained with papain compare to the one obtained with alcalase. (Li et al., 2012).

Antihypertensive activity

ACE inhibitors prevent the occurrence of high blood pressure by inhibiting the enzyme that catalyzes the conversion of angiotensin I to angiotensin II, which is a vasoconstrictor (Lee et al., 2010). In our study, the enzymatic hydrolysates showed ACE inhibition potential, whose values varied between 50.75-63.65% (Fig. 3). The enzymatic hydrolysate obtained with alcalase showed a slightly higher degree of ACE inhibition than that obtained with papain. Similar results were reported by Lee et al. (2010) for the hydrolysates obtained by treating the tuna skeleton with several enzymes, including papain and alcalase, observing that alcalase hydrolysate had a slightly higher antihypertensive activity than papain hydrolysate. As in our case, the degree hydrolysis of the alcalase extract was higher than that of papain, and this may influence the antihypertensive activity (Lee et al., 2010). Also Choonpicharn et al. (2015) found that gelatin hydrolysate from Nile tilapia skin obtained with alcalase exhibited an increased antihypertensive activity than papain hydrolysate.

Biocompatibility and antiproliferative activity

The results of biocompatibility test performed in normal fibroblast cells (NCTC cell line) have showed variable cell viability when cultivated in the presence of protein hydrolysates, depending on the samples concentration (3-6 mg/ml) (Fig. 4). In the case of papain hydrolysate, the values of cell viability were higher than 80% for all tested concentrations. The flavourzyme hydrolysate was cytocompatible (cell viability over 80%) at concentrations ranging between 3-6 mg/ml, and induced a decrease of cell viability down to 58.5% at a concentration of 10 mg/ml protein hydrolysate in the culture media. Similar variation was observed in the case of alcalase hydrolysate, but the cell viability values decreased to 73.94 and 48.7%, at 8 and 10 mg/ml, respectively, the last value representing the lowest viability in the experiment. The flavourzyme and alcalase hydrolysate induced an increase of the

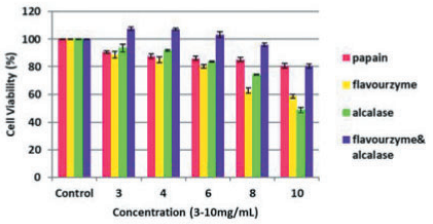

Figure 4. Cell viability of fibroblast cells (NCTC cell line) after 48 h of cultivation in standard conditions, in the presence of enzymatic hydrolysates. The results are expressed as mean ± SD (n=3).

cell viability at concentrations between 3-6 mg/ml, showing stimulation of cell metabolism. Thus, the values of cell viability (103.12%-107.41%) were higher than that in the control sample (100%). At higher concentrations, the cell viability decreased down to 80.5%. All hydrolysates were cytocompatible, excepting the flavourzyme and alcalase extracts, at 8-10 mg/mL.

The evaluation of the antiproliferative activity was performed in a stabilized line of Hep-2 tumor cells in the presence of different samples concentrations (3-10 mg/ml). Antiproliferative activity of the analyzed fish protein hydrolysates increased proportionally with their concentration, thus reaching the maximum value at the concentration of 10 mg/ml. The protein hydrolysates obtained by combined treatment with flavourzyme and alcalase applied in the culture of Hep-2 cancer cells, at 10 mg/ml concentration, showed lower cell viability (29.58%) than the identical treatment in NCTC cell culture (80.5%) (Fig. 5).

The antiproliferative character was also observed in the case of papain extract at 10 mg/ml concentration, because the cell viability of Hep-2 cells was 39.8%, while in NCTC cells identically treated was 80.7%. In the case of hydrolysates obtained with separate alcalase and flavourzyme, the viability of Hep-2 cells was 34.7% and 47.2% at 10 mg/ml concentrations, but they were cytotoxic for normal NCTC cells. Thus, the protein hydrolysates obtained with papain and combined flavourzyme-alcalase treatment had antiproliferative activity in Hep-2 tumor cells.

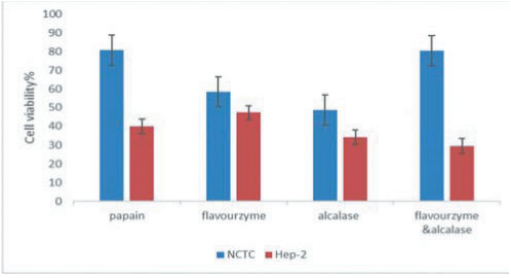

Figure 5. Cell viability of normal (NCTC cell line) and tumoral cells (Hep-2 cell line) after 48 h of cultivation in standard conditions, in the presence of enzymatic hydrolysates, at a concentration of 10 mg/ml. The results are expressed as mean ± SD (n=3).

Previous studies on the bioactive properties of roe or fish peptides showed their antiproliferative effect. Half-fin anchovy pepsin hydrolysate had antiproliferative effect on DU-145 human prostate cancer cell line, 1299 human lung cancer cell line and 109 esophagus cancer cell line (Song et al., 2011). Rohu roe pepsin hydrolysate showed antiproliferative effect against Caco-2 cells (Chalamaiah et al., 2015). Two peptides obtained from tuna dark muscle using papain and protease XXIII showed antiproliferative effect on human breast cancer cell line MCF-7 (Zamora-Sillero, 2018).

Conclusions

The protein hydrolysates obtained from silver carp tissue waste (skin, bones, meat) by enzymatic treatment with papain, flavourzyme and alcalase demonstrated important bioactive properties, including antioxidant, antihypertensive and antiproliferative activity. The study recommends their further analysis as ingredients of novel valuable nutraceuticals.

Acknowledgements

This work was supported by a grant of the Romanian Ministry of Education and Research, CCCDI - UEFISCDI, project number PN-III-P2-2.1-PTE-2019-0181, within PNCDI III

References

1. Adler-Nissen J. Determination of the degree of hydrolysis of food protein hydrolysates by trinitrobenzenesulfonic acid. *Journal of Agricultural and Food Chemistry. 1979;27(6): 1256–1262.* doi:10.1021/jf60226a042
2. Chalamaiah M, Dinesh K, Hemalatha R, Jyothirmayi T. Fish protein hydrolysates: Proximate composition, amino acid composition, antioxidant activities and applications: A review. *Food Chemistry. 2012; 135:* 3020–3038
3. Chalamaiah M, Jyothirmayi T, Diwan PV, Dinesh K, Antiproliferative, ACE-inhibitory and functional properties of protein hydrolysates from rohu (Labeo rohita) roe (egg) prepared by gastrointestinal proteases. *Journal of Food Science and Technology.* 2015; 52(12):8300–8307 doi 10.1007/s13197-015-1969
4. Choonpicharn S, Jaturasitha S, Rakariyatham N, Suree N, Niamsup H. Antioxidant and antihypertensive activ-

ity of gelatin hydrolysate from Nile tilapia skin. *Journal of Food Science and Technology.*2015; 52(5):3134–3139 doi 10.1007/s13197-014-1581-6

5. Gao R, Yu Q, Shen Y, Chy Q, Chen G, Fen S, Yang M, Yuan L, McClements DV, Sun Q. Production, bioactive properties, and potential applications of fish protein hydrolysates: Developments and challenges. *Trends in Food Science & Technology.* 2021: 110:687-699
6. Idowu AT, Benjakul S, Sinthusamran S, Sookchoo P, Kishimura H. Protein hydrolysate from salmon frames: Production, characteristics and antioxidative activity. *Journal of Food Biochemistry. 2018;* doi : 10.1111/jfbc.12734
7. Je JY, Qian ZJ, Byun HG, Kim SK. Purification and characterization of an antioxidant peptide obtained from tuna backbone protein by enzymatic hydrolysis. *Process Biochemistry.* 2007; 42: 840-846
8. Jia J, Zhou Y, Lu J, Chen A, Li Y, Zheng G. Enzymatic hydrolysis of Alaska pollack (Theragra chalcogramma) skin and antioxidant activity of the resulting hydrolysate. *Journal of the Science of Food and Agriculture.* 2010; 90: 635–640
9. Lee SH, Qian ZJ, Kim SK. A novel angiotensin I converting enzyme inhibitory peptide from tuna frame protein hydrolysate and its antihypertensive effect in spontaneously hypertensive rats. *Food Chemistry.* 2010*;* 118:96-102
10. Li X, Luo Y, Shenb H, Youa J. Antioxidant activities and functional properties of grass carp (Ctenopharyngodon idellus) protein hydrolysates. *Journal of the Science of Food and Agriculture*. 2012; 92:292-298
11. Noman A, Xua Y, Al-Bukhaiti W, Abed S, Ali A, Rhamadhan A, Xia W. Influence of enzymatic hydrolysis conditions on the degree of hydrolysis and functional properties of protein hydrolysate obtained from Chinese sturgeon (Acipenser sinensis) by using papain enzyme. *Process Biochemistry.* 2018; 67: 19-28
12. Roslan J, Yunos KF, Abdullah N, Kamala MM. Characterization of Fish Protein Hydrolysate from Tilapia (Oreochromis niloticus) by-Product. *Agriculture and Agricultural Science Procedia.* 2014; 2:312-319
13. Papadimitriou CG, Vafopoulou-Mastrojiannaki, A, Silva SV, Gomes, AM, Malcata FX, Alichanidis E. Identification of peptides in traditional and probiotic sheep milk yoghurt with angiotensin I-converting enzyme (ACE)-inhibitory activity. *Food Chemistry.* 2012; *105: 647- 656*.
14. Quetin-Leclercq J., Elias R., Balansard G. Cytotoxic activity of some triterpenoid saponins, *Planta Med*.1992; 58: 279-280
15. Schägger H, Tricine SDS-PAGE. *Nature protocols*. 2006;1:16-23
16. Tacias Pascacio V, Castaneda-Valbuena D, Morellon-Sterling R, Tavano O, Berenguer-Murcia A. Bioactive peptides from fisheries residues: A review of use of papain in proteolysis reactions. *International Journal of Biological Macromolecules.* 2021; 184:415-428
17. Zamora-Sillero J, Gharsallaoui A, Prentice C. Peptides from Fish By-product Protein Hydrolysates and Its Functional Properties: an Overview. *Marine Biotechnology.* 2018; 20:118–130
18. Song R, Wei R, Zhang B, Yang Z, Wang D. Antioxidant and Antiproliferative Activities of Heated Sterilized Pepsin Hydrolysate Derived from Half-Fin Anchovy (Setipinna taty). *Marine Drugs.* 2011; 9: 1142-1156; doi:10.3390/md9061142
19. Zhang L, Shan Y, Hong H, Luo Y,Hong XYea W. Prevention of protein and lipid oxidation in freeze-thawed bighead carp (Hypophthalmichthys nobilis) fillets using silver carp (Hypophthalmichthys molitrix) fin hydrolysates. *LWT - Food Science and Technology*. 2020; 123: 109050
20. Zhang X, Dai Z, ZhangY, DongY, Hu X, Structural characteristics and stability of salmon skin protein hydrolysates obtained with different proteases, *LWT - Food Science and Technology*. 2021; doi: https://doi.org/10.1016/j.lwt.2021.112460.
21. Zheng K, Wu L, He Z, Yang B, Yang Y. Measurement of the total protein in serum by biuret method with uncertainty evaluation. *Measurement*. 2017; 112: 16–21
22. Zhong S, Ma C, Lin Y, Luo Y, Antioxidant properties of peptide fractions from silver carp (Hypophthalmichthys molitrix) processing by-product protein hydrolysates evaluated by electron spin resonance spectrometry. *Food Chemistry*. 2011; 126:1636-1642
23. Wang K, Han L, Hong H, Pan J, Liu H, Luo Y. Purification and identification of novel antioxidant peptides from silver carp muscle hydrolysate after simulated gastrointestinal digestion and transepithelial transport. *Food Chemistry.* 2021; 342: 128275.