Journal of Food Chemistry & Nanotechnology

https://doi.org/10.17756/jfcn.2018-055

Research Article

Open Access

A Comparative Study on Histamine Levels of Refrigerated Trout Fillets Using Competitive ELISA and HPLC Methods

Hanieh Amani[1], Mohammad Hassan Kamani[2*], Omid Safari[3], Farzane Vakilchap[4] and Masoomeh Mehraban Sang Atash[5]

[1]*Department of Food Science and Technology, Sabzevar Branch, Islamic Azad University, Sabzevar, Iran*
[2]*Young Researchers and Elite Club, Sabzevar Branch, Islamic Azad University, Sabzevar, Iran*
[3]*Department of Fishery, Faculty of Natural Resources and Environment, Ferdowsi University of Mashhad, Mashhad, Iran*
[4]*Department of Biotechnology, Tarbiat Modares University, Tehran, Iran*
[5]*Department of Food Quality and Safety, Food Science and Technology Research Institute, ACECR Mashhad Branch, Mashhad, Iran*

[*]Correspondence to:
Mohammad Hassan Kamani
Young Researchers and Elite Club
Sabzevar Branch, Islamic Azad University
Sabzevar, Iran
Tel:+98-937-4342212, +989374342212
Fax:+98-571-2647513
E-mail: mohammadh.kamani@gmail.com

Received: May 13, 2018
Accepted: July 03, 2018
Published: July 05, 2018

Citation: Amani H, Kamani MH, Safari O, Vakilchap F, Atash MMS. 2018. A Comparative Study on Histamine Levels of Refrigerated Trout Fillets Using Competitive ELISA and HPLC Methods. *J Food Chem Nanotechnol* 4(2): 33-37.

Published by United Scientific Group

Abstract

Histamine is one of the well-known biogenic amines and responsible for causing allergic reactions. In the present study, the level of histamine measured in the refrigerated stored (+4 °C) fillets of rainbow trout (*Oncorhynchus mykiss*) during time intervals (0, 5, 10, 15 and 20 days) using immunological (ELISA) and analytical (HPLC) methods. During the storage time, the histamine content in all samples significantly increased (p<0.05), while none of sample exceed the allowable limit of 100 mg.kg^{-1}. Moreover, there was significant correlation coefficients (r = 89.1) between HPLC and ELISA method (p<0.05). The regression models showed the quadratic models has superiority than linear in predicting histamine content. Overall, the present study revealed that ELISA method could be used to detect the low level of histamine as compared to HPLC method. Therefore, in view of the accuracy obtained for ELISA method, it may be considered as appropriate method for measuring low quantities of histamine in fish flesh.

Keywords

Histamine, Fillet, Competitive ELISA method, HPLC, Regression, Quality

Nomenclature

HPLC: High Performance Liquid Chromatography; ELISA: Enzyme-linked Immunosorbent Assay; GC: Gas Chromatography; TLC: Thin Layer Chromatography; FIA: Flow Injection Analysis; DAY: Storage time; HIS: Histamine; ANOVA: Analysis of Variance; r: Coefficient of Correlation; $R^2_{adjusted}$: Modified R-square

Introduction

Fish is considered as a rich source of quality proteins and polyunsaturated fatty acids, which are essential parts of the human diet [1]. Due to the presence of these sensitive components, fish flesh is considered to be a highly perishable food to spoilage incident. The quality of flesh may reduce by various complex incidents such as enzymatic, biochemical and microbial reactions [2]. In over recent years, the consumer's preference for the use of fresh fish has been remarkably increasedand, therefore accurate quality control is now more critical [3, 4].

Biogenic amines group is known as a quality factor in the reducing the freshness of the fish flesh. They are derived from decarboxylation of amino

acids during spoilage process. The most important biogenic amines in fish and their amino acids responsible for them are tyramine (tyrosine), cadaverine (lysine), histamine (histidine), agmatine (arginine), tryptamine (tryptophan), putrescine (ornithine), beta phenyl ethyl amine (phenylalanine), serotonin (tryptophan), spermine, and spermidine [5-7]. Among of, histamine or HIS [1H-imidazole-4-ethanamine] is one of the major toxins, which is produced from the bacterial decomposition of histidine amino acid by the histidine decarboxylase enzyme (Figure 1). It is a small organic mono-alkaline molecule with a molecular weight of 111.15 g.mol[1] and its production is directly associated with the ability of the microorganism in producing the histidine decarboxylase enzyme. The increasing level of HIS content in seafood is highly linked with reducing the quality of fish flesh [8, 9].

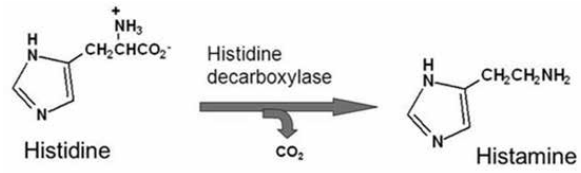

Figure 1: Histidine metabolism and formation of histamine toxin during bacterial decarboxylation process.

Thus far, several methods have been introduced to estimate the HIS content. All of these methods are divided into the quantitative and semi-quantitative categories [10]. Among the HPLC [11], TLC [12] and ELISA [13] techniques have been gained more attention. Various studies have been carried out to compare the quality, accuracy and reliability of these methods in different food items. The comparative study on TLC and HPLC procedures in the determination of HIS content in fresh fish by Jeya Shakila et al.[14] and the comparison of the HPLC and ELISA procedures in the measuring the HIS content in wine by Marcobal et al. [15], can be regarded as two prime examples.

The rainbow trout is widely recognized as an important part of food basket in the developing countries [16]. Due to low level of histidine amino acid content in rainbow trout, the amount of HIS is usually low and as a consequence, its detection and estimation are little difficult compared to other fishes such as Scombroid species which are rich in histidine. Therefore, evaluation of the validity of current methods is needed in order to find the best method with the highest accuracy for measuring HIS content in rainbow trout. In addition, the storage time may highly affect the content of HIS toxin especially in refrigerated condition. Therefore, this study aimed to focus on 1) effect of storage time on the HIS content of rainbow trout under cold condition; and 2) to compare the efficiency of immunological (ELISA) and analytical (HPLC) methods in tracing HIS at low concentrations in fish flesh.

Materials and Methods

Preparation of samples

The rainbow trout (with average weights 600 ± 50 g) were purchased from a fish farm in Mashhad (Khorasan Razavi Province, Iran) and then transported to the laboratory of ACECR located in Mashhad city under refrigerated controlled condition. Afterward, the fish were beheaded, cleaned and well washed with cold water and finally, the fillets were prepared in uniform dimensions. The samples then packed in polyethylene packages and preserved for 0, 10, 15, and 20 days in refrigerator for subsequent HIS analysis.

Determination of HIS content

Determination of HIS by immunological analysis

The RIDASCREEN® kit was procured from the R-Bipharm Co. (Darmstadt, Germany) and used according to the recommended protocol which described by the company.

Sample preparation for ELISA kit

To prepare the sample, one gram homogenized flesh was dissolved in 9 mL distilled water and then centrifuged for 5 min at 2500 g. 1 mL of the supernatant beneath the fat layer removed and diluted with 9.8 mL distilled water, and then 100 µL of this final solution used for the next step. To prepare the buffer washing solution, concentrated buffer washing solution was diluted in distilled water at the ratio of 1:49, followed by storing at +2 ˚C for the subsequent step.

Histamine analysis by the ELISA kit

After the sample preparation, the HIS was converted to N-acylhistamine by an acylation reagent. After washing, the secondary peroxidase-conjugated antibodies (enzyme conjugate) are added. These antibodies bind to the antibody-histamine complex; then, unbound antigen is removed by washing. Afterwards, the substrate and chromogen (tetramethyl-benzidine) are added into wells of the micro-titration plate and then are incubated. During incubation, the bound enzyme conjugation converted a colourless chromogen into blue product, and blue color changed into yellow after addition of stop solution. After the substrate reaction, the light absorption was measured at 450 nm using a microplate reader (EL-808, BIO-TEK Instruments Inc., USA). The HIS content determined from the standard graph plotted via respective software and reported in $mg.kg^{-1.}$

Determination of histamine by High-Performance Liquid Chromatography

After homogenizing two fillets from each package with 10 mL trichloroacetic (TCA 5%) using a laboratory blender (Abzar Sazan Co., Isfahan, Iran), 75 g minced flesh were centrifuged (Heraeus Sepatech Gmbh, Labofuge, Germany) at 4000 rpm for 10 min and the supernatant was removed. The extraction step was repeated three times on the remaining materials with 10 mL TCA. After TCA extraction, derivatization were done with benzoyl chloride according to Rezaei et al. [16]. Afterwards, 1 ml NaOH (2 M), 5 µl benzoyl chloride and 2 ml NaOH were added in order to stop derivatization, and followed by centrifuging at 2500 rpm for 5 min at +4 ˚C. The supernatants were dried and 200 µL methanol was added on the dried material. Finally, the mixture passed through a filter (0.45 µm) and 20 µL of filtrate injected to the HPLC apparatus. The analyses carried out using a High-performance liquid chromatography

(HPLC) (Waters 1525; Waters Company, Milford, MA, USA) equipped with a UV-detector Waters 2487 set at 254 nm. The column was reversed phase, C18 Waters Spherisorb ODS-2 (250×4.60 mm; particle diameter, 5 µm) which was supplied Waters Spherisorb pre-column cartridge (10 mm, length) packed with the same material and both of them were bought from Waters Co. (Milford, MA, USA). The chemicals used including histamine standard, methanol, chloroform, butanol, diethyl ether and n-heptane, benzoyl chloride, HCl, NaCl, and NaOH were purchsed from the Fluka and Merck companies. The content of HIS was reported as $mg.kg^{-1}$.

Statistical analysis

One-way ANOVA was used to compare the means in a completely randomized design. Duncan's multiple range test was applied to compare the significant differences among means ($p<0.05$) using SPSS™ software version 19 (SPSS Inc., Chicago, IL, USA). The changes in the HIS contents during storage time were evaluated using regression analyses and the coefficient of determination separately calculated for each equation. All measurements were carried out in triplicate and results were reported as mean ± SD.

Results and Discussion

The HIS content determined by the HPLC & ELISA methods and its variations were recorded during storage times. As shown in figure 2, the results obtained from both methods indicated an increasing trend in HIS content of the rainbow trout fillet during storage times ($p<0.05$). The initial HIS content recorded by ELISA and HPLC to be 1.2 $mg.kg^{-1}$ and 1.96 $mg.kg^{-1}$, respectively. This amount significantly increased on day 10 for the ELISA and on day 15 for the HPLC method. The highest level of HIS was found to be 10.6 $mg.kg^{-1}$ on day 20 which detected by ELISA method and was significantly different compared to the previous days ($p<0.05$).

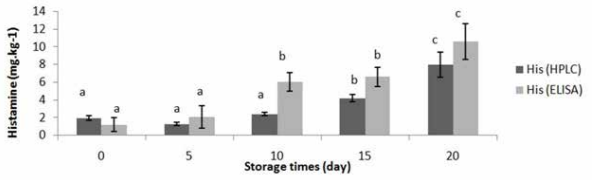

Figure 2: Mean ± standard deviation for the histamine content ($mg.kg^{-1}$) in rainbow trout fillets during storage times.

The linear and quadratic regression analyses were carried out to mathematically predict the change of HIS during the storage time. Different models were built which are as follows (equations 1- 4):

$$His = 0.58 + 0.299\ DAY \quad (R^2_{adjusted} = 69.4;\ P < 0.05)$$
(Eq.1: HPLC method)

$$His = 1.94 - 0.24\ DAY + 0.027\ DAY^2 \quad (R^2_{adjusted} = 98.81;\ P < 0.05)$$
(Eq. 2: HPLC method)

$$His = 0.63 + 0.46\ DAY \quad (R^2_{adjusted} = 92.7;\ P < 0.05)$$
(Eq. 3. ELISA method)

$$His = 1.043 + 0.303\ DAY + 0.008\ DAY^2 \quad (R^2_{adjusted} = 91.1;\ P < 0.05)$$
(Eq. 4 ELISA method)

In figure 3 and 4, the plots of linear and quadratic models are depicted. The regression analysis revealed a good coefficient for the obtained models. Therefore, these models may possibly be useful to predict the HIS content and quality of rainbow trout fillet during the storag time. To compare the obtained results from HPLC and ELISA, the coefficient of correlation between two procedures was also determined. The results demonstrated that the amount of coefficient of correlation was 89.1, indicating an acceptable value and validity for both methods (Figure 5).

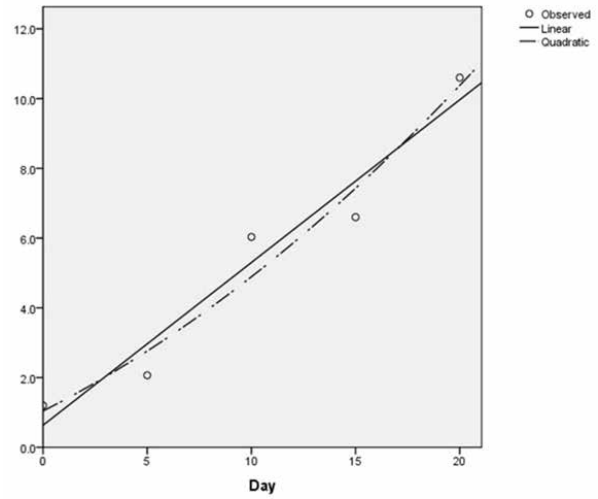

Figure 3: Linear and quadratic regression analyses for histamine content of rainbow trout during cold storage times (method of analysis: ELISA).

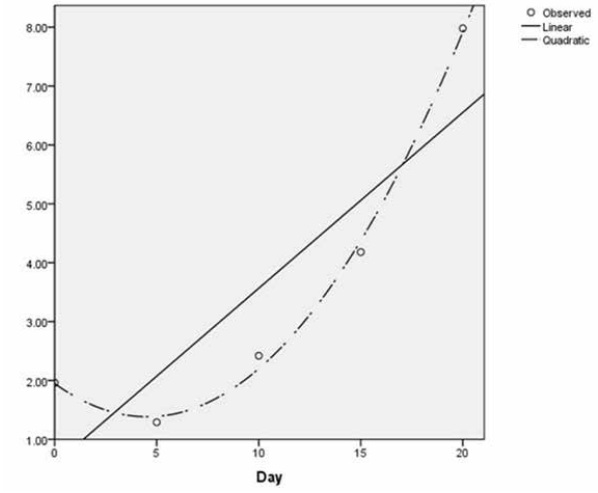

Figure 4: Linear and quadratic regression analyses for histamine content of rainbow trout during cold storage times (method of analysis: HPLC).

HIS is one of the most active biogenic amines, which is mainly produced from the bacterial decarboxylation of histidine amino acid and releases by proteolysis [17]. A significant increasing trend observed in the HIS content in all samples at different time intervals. The initial HIS content by HPLC and ELISA procedures were found to be 1.96 $mg.kg^{-1}$ and

1.2 mg.kg^{-1}, respectively, which increased after 20 days refrigeration to 7.98 mg.kg^{-1} and 10.6 mg.kg^{-1}. Different amounts for the initial and final HIS concentrations have been reported in the literature. Matejkova et al. [18] reported zero and 17.5 mg.kg^{-1} HIS contents for initial and final concentration *Oncorhynchus mykiss* after 28 days refrigerated storage. However, the other study reported 1.6 mg.kg^{-1} for this toxin in the same fish after 18 days cold storage [16]. This difference between the initial and final amount can be dependent upon various factors such as the initial concentration of histidine amino acid, the extent of bacterial growth and activity, storage conditions as well as temperature [19, 20]. In this study, none of the samples exceed the maximum allowable limit for HIS, which is set at 100 mg.kg^{-1} [21].

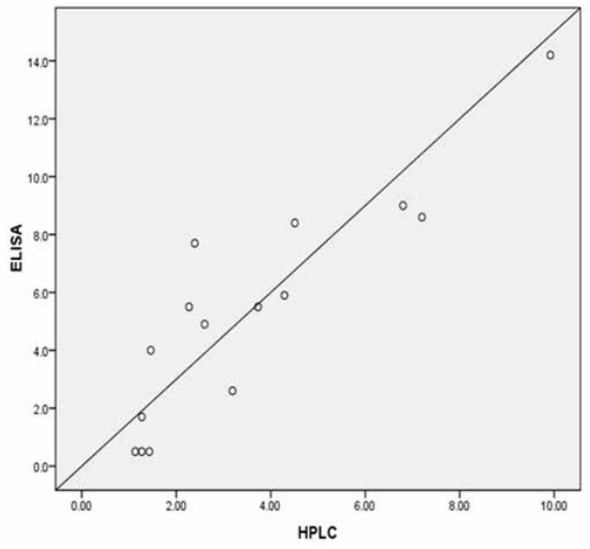

Figure 5: Comparison of the results of histamine content in the rainbow trout fillet by the HPLC & ELISA methods (r^2 = 89.1).

Decarboxylase activity in seafood products and the production of amines like HIS is mainly performed by the *Enterobacteriaceae*, *Pseudomonas*, *Micrococcus*, and Lactic bacteria groups [22] which have a higher histological potential than other microorganisms in the aerobic conditions [23]. It has been documented that the activity of microorganisms such as *Morganella*, *Escherichia coli*, *Enterobacter*, and *Proteus vulgaris* is the main reason for the increase of HIS content. The existing histidine in the muscles is affected by the decarboxylase histidine enzyme as a substrate and HIS is subsequently produced [13, 24]. HIS is non-volatile and is not destroyed by cooking and causes scombroid poisoning in the consuming individual. This is a mild illness with symptoms such as urticaria, nausea, vomiting, and diarrhea [25]. Many factors such as pH, water activity, salt concentration, and additives may influence the rate of microorganism activities, which are involved in the producing these biogenic amines. Among all environmental factors, the storage temperature is considered as the most influential factor [17].

According to HACCP regulations, HIS can be used as a qualitative parameter to evaluate the freshness of fish [16]. Although a long storage time (20 days) used in this study, the HIS content was still below the allowable limit (100 mg.kg^{-1}). Some studies also reported either negligible amounts or no trace of this toxin in the samples during a twelve-day preservation period [26]. Therefore, it can be concluded that HIS may not possibly be a suitable qualitative parameter for all fish species and is better to consider the other chemical and bacterial parameters for assessing the freshness.

Nowadays, various methods such as colorimetric, TLC, enzymatic, amino enzymatic and FIA are used for measuring biogenic amines [10]; however, HPLC and GC chromatography are the most popular methods [27]. The ELISA procedure is a special and sensitive method, which was developed by Serrar et al. [10] to measure the HIS content. This method is based on the competition between the antigen and the antibody and results are obtained from the standard graph which for the ELISA procedure. Quickness and simultaneous analysis of several samples, reduction of incubation time, and simple application are some of the advantages of this method [10, 15]. According to Figure 2, the results of ELISA method indicated a higher growth in HIS in comparison with HPLC procedure, and the coefficient of correlation between the two procedures was determined 89.1 (Figure 5). The HIS amount was significantly increased on day 10 in the ELISA procedure, while for the value HIS by HPLC did not happen any change. This shows that there is some slight difference in detected HIS content by these two methods viz., HPLC and ELISA, however, the final results are closed to each other. As can be seen in figure 2 the standard deviation in the HPLC is lower than that in the ELISA method, representing the high precision of the HPLC procedure.

By considering the advantages and the disadvantages of these two procedures, it can be concluded that the implementation of ELISA method is at first recommended and the HPLC should only be used when the need for more precise detection amount of HIS. Similar to the present research, different comparative studies have been carried out to evaluate the accuracy of HPLC and ELISA in detection of HIS in various foodstuffs such as canned fish with a coefficient r = 0.97 [28], Spanish wine a coefficient of correlation r = 0.91 [15], commercial doenjang and gochujang with a coefficient of correlation r = 0.969 [29], and cheese with a coefficient of correlation r = 0.979 [30]. The results of these studies are revealed that there is an acceptable difference between these two procedures and are in a good agreement with above findings.

Conclusion

The rainbow trout is considered to be one of the perishable foods due to the presence of sensitive amino acids and significant amounts of long-chain fatty acids. HIS is a toxin, which is formed by the decarboxylation of amino acid and is counted as a freshness parameter in fish. The HIS content is measured by various methods. In this research, the HIS content of the rainbow trout fillet was determined during time intervals 0, 5, 10, 15, and 20 days by the HPLC and ELISA procedures under cold storage condition. The HIS content in all samples significantly increased but none exceed the allowable limit of 100 mg.kg^{-1}. The correlation

coefficient between the two procedures was found to be high with the value 89.1. By considering the advantages and the disadvantages of both procedures, it can be concluded that the use of ELISA method is at first recommended and the HPLC should only be used when the need for more precise detection amounts of HIS.

Acknowledgments

The authors wish to thank the staff of veterinary laboratory in Khorasan Razavi Province (Mr. Nowruzi) for their technical support.

References

1. Pezeshk S, Hosseini H, Rezaei M, Khaksar R. 2013. Evaluation of shelf life of live and gutted fish treated with a shallot extract. *J Food Process Pres* 37(5): 970-976. https://doi.org/10.1111/j.1745-4549.2012.00765.x
2. Pezeshk S, Rezaei M, Hosseini H. 2011. Effects of turmeric, shallot extracts, and their combination on quality characteristics of vacuum-packaged rainbow trout stored at 4 ± 1 °C. *J Food Sci* 76(6): M387-M391. https://doi.org/10.1111/j.1750-3841.2011.02242.x
3. Kilinc B, Cakli S, Dincer T, Tolasa S. 2009. Microbiological, chemical, sensory, color, and textural changes of rainbow trout fillets treated with sodium acetate, sodium lactate, sodium citrate, and stored at 4 °C. *Journal of Aquatic Food Product Technology* 18(1-2): 3-17. https://doi.org/10.1080/10498850802580924
4. Barat JM, Gallart-Jornet L, Andre´s A, Akse L, Carlehog M, et al. 2006. Influence of cod freshness on the salting, drying and desalting stages. *J Food Eng* 73(1): 9-19. https://doi.org/10.1016/j.jfoodeng.2004.12.023
5. Halasz A, Barath A, Simon-Sarkadi L, Holzapfel W. 1994. Biogenic amines and their production by microorganisms in food. *Trends Food Sci Technol* 5(2): 42-49. https://doi.org/10.1016/0924-2244(94)90070-1
6. Onal A. 2007. A review: current analytical methods for the determination of biogenic amines in foods. *Food Chem* 103(4): 1475-1486. https://doi.org/10.1016/j.foodchem.2006.08.028
7. Shalaby A. 1996. Significance of biogenic amines to food safety and human health. *Food Res Int* 29(7): 675-690. https://doi.org/10.1016/S0963-9969(96)00066-X
8. Du WX, Lin CM, Phu AT, Cornell JA, Marshall MR, et al. 2002. Development of biogenic amines in yellowfin tuna (*Thunnus albacares*): effect of storage and correlation with decarboxylase positive bacterial flora. *J Food Sci* 67(1): 292-301. https://doi.org/10.1111/j.1365-2621.2002.tb11400.x
9. Ozogul Y, Özogul F, Kuley E, Özkutuk AS, Gökbulut C, et al. 2006. Biochemical, sensory and microbiological attributes of wild turbot (*Scophthalmus maximus*), from the Black Sea, during chilled storage. *Food Chem* 99(4): 752-758. https://doi.org/10.1016/j.foodchem.2005.08.053
10. Etienne M. 2006. Methodology for histamine and biogenic amines analysis. Seafoodplus Traceability. Project 6.3.
11. Park JS, Lee CH, Kwon EY, Lee HJ, Kim JY, et al. 2010. Monitoring the contents of biogenic amines in fish and fish products consumed in Korea. *Food Control* 21(9): 1219-1226. https://doi.org/10.1016/j.foodcont.2010.02.001
12. Lapa-Guimarães J, Pickova J. 2004. New solvent systems for thin-layer chromatographic determination of nine biogenic amines in fish and squid. *J Chromatogr A* 1045(1-2): 223-232. https://doi.org/10.1016/j.chroma.2004.06.014
13. Rahimi E, Nayebpour F, Alian F. 2012. Determination of histamine in canned tuna fish using ELISA method. *American-Eurasian Journal of Toxicological Sciences* 4(2): 64-66. https://doi.org/10.5829/idosi.aejts.2012.4.2.56188
14. Jeyashakila R, Vasundhara TS, Kumudavally KV. 2001. A comparison of the TLC-densitometry and HPLC method for the determination of biogenic amines in fish and fishery products. *Food Chem* 75(2): 255-259. https://doi.org/10.1016/S0308-8146(01)00173-X
15. Marcobal A, Polo MC, Martın-Alvarez PJ, Moreno-Arribas MV. 2005. Biogenic amine content of red Spanish wines: comparison of a direct ELISA and an HPLC method for the determination of histamine in wines. *Food Res Int* 38(4): 387-394. https://doi.org/10.1016/j.foodres.2004.10.008
16. Rezaei M, Montazeri N, ErshadLangrudi H, Mokhayer B, Parviz M, et al. 2007. The biogenic amines and bacterial changes of farmed rainbow trout (*Oncorhynchus mykiss*) stored in ice. *Food Chem* 103(1): 150-154. https://doi.org/10.1016/j.foodchem.2006.05.066
17. Visciano P, Schirone M, Tofalo R, Suzzi G. 2012. Biogenic amines in raw and processed seafood. *Front Microbiol* 3: 188. https://doi.org/10.3389/fmicb.2012.00188
18. Matejkova M, Krizek M, Vacha F, Dadakova E. 2013. Effect of high-pressure treatment on biogenic amines formation in vacuum-packed trout flesh (*Oncorhynchus mykiss*). *Food Chem* 137(1-4): 31-36. https://doi.org/10.1016/j.foodchem.2012.10.011
19. Rehbein H, Oehlenschlager J. 2009. Fishery product quality, safety and authenticity. Blackwell Publishing Ltd, United Kingdom. https://doi.org/10.1002/9781444322668
20. Ozogul Y, Ozogul F, Gokbulut C. 2006. Quality assessment of wild European eel (*Anguilla anguilla*) stored in ice. *Food Chem* 95(3): 458-465. https://doi.org/10.1016/j.foodchem.2005.01.025
21. Křížek M, Matějková K, Vácha F, Dadáková E. 2012. Effect of low-dose irradiation on biogenic amines formation in vacuum-packed trout flesh (*Oncorhynchus mykiss*). *Food Chem* 132(1): 367-372. https://doi.org/10.1016/j.foodchem.2011.10.094
22. Ruiz-capillas C, Jimenez-colmenero F. 2004. Biogenic amines in meat and meat products. *Crit Rev Food Sci Nutr* 44(7-8): 489-499. https://doi.org/10.1080/10408690490489341
23. Pons-Sa´nchez-Cascado S, Veciana-Nogue´s MT, Bover-Cid S, Marine´-Font A, Vidal-Carou MC. 2006. Use of volatile and non-volatile amines to evaluate the freshness of anchovies stored in ice. *J Sci Food Agric* 86(5): 699-705. https://doi.org/10.1002/jsfa.2398
24. Bremner HA. 2002. Safety and quality issues in fish processing. CRC Press LLC, USA.
25. Chen H, Huang Y, Hsu H, Lin C, Chen W, et al. 2010. Determination of histamine and biogenic amines in fish cubes (*Tetrapturus angustirostris*) implicated in a foodborne poisoning. *Food Control* 21(1): 13-18. https://doi.org/10.1016/j.foodcont.2009.03.014
26. Rodríguez CJ, Besteiro I, Pascual C. 1999. Biochemical changes in freshwater rainbow trout (*Oncorhynchus mykiss*) during chilled storage. *J Sci Food Agric* 79(11): 1473-1480. https://doi.org/10.1002/(SICI)1097-0010(199908)79:11<1473::AID-JSFA389>3.0.CO;2-C
27. Patang SB, Mukundan MK, AshokKumar K. 2005. A simple and rapid method for colorimetric determination of histamine in fish flesh. *Food Control* 16(5): 465-472. https://doi.org/10.1016/j.foodcont.2004.05.008
28. Muscarella M, Lo Magro S, Campaniello M, Armentano A, Stacchini P. 2012. Survey of histamine levels in fresh fish and fish products collected in Puglia (Italy) by ELISA and HPLC with fluorimetric detection. *Food Control* 31(1): 211-217. https://doi.org/10.1016/j.foodcont.2012.09.013
29. Kim TK, Lee JI, Kim JH, Mah JH, Hwang HJ, et al. 2011. Comparison of ELISA and HPLC methods for the determination of biogenic amines in commercial doenjang and gochujang. *Food Sci Biotechnol* 20(6): 1747-1750. https://doi.org/10.1007/s10068-011-0241-0
30. Aygün O, Schneider E, Scheuer R, Usleber E, Gareis M, et al. 1999. Comparison of ELISA and HPLC for the determination of histamine in cheese. *J Agri Food Chem* 47(5): 1961-1964. https://doi.org/10.1021/jf980901f