Development of a new method for extracting histamine from marine fish flesh using the salting-out technique

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Abstract

A simple and practical method was developed to extract histamine from fish products using sodium chloride (NaCl). After obtaining a saline extract from fish samples, histamine was derivatized by a condensation reaction with ortho-phthalaldehyde. Fluorescence intensity was measured by a fluorimeter. The first part of this work concerned a solid-liquid extraction tested with samples from the food analysis performance assessment scheme. The best histamine extraction yield (97%) was obtained using an extraction time of 4 minutes, a temperature of 40°C, and a NaCl/water ratio of 41% (w/w). The second part focused on a liquid-liquid extraction carried out on standard solutions of histamine (45, 90, and 180 mg/kg). The use of NaCl (41%) and trichloroacetic acid [(TCA) 10%] did not show any significant difference in extraction yield. The yield obtained was 99.15-100.1% for TCA (10%) and 98.65-99.45% for NaCl (41%). The validation criteria (repeatability and reproducibility) were checked by evaluating the reliability of the method. Extraction using NaCl has proven to be an interesting alternative method for the extraction of histamine from fish, as it is reliable, inexpensive, and less hazardous.

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Introduction

Histamine is a biogenic amine produced following the decarboxylation of histidine by bacterial and tissue enzymes (FDA, 2020). It is produced in a significant way in food; in particular, fish products can include fresh, processed, and other items directly obtained from fish when the high levels of free histidine content of the fish and the presence of bacteria capable of synthesizing histidine decarboxylase, in addition to the storage and hygiene conditions, are not adequate. It is the leading cause of food poisoning worldwide (Biji *et al*., 2016). Since histamine is a chemical hazard of food origin, determining its level is essential for food chemistry to better control food safety. Methods such as colorimetry, fluorometry, enzyme immunoassay, and chromatography have been developed over the last decades for the determination of histamine (Prester, 2011; Bilge and Hüseyin, 2015). All of these methods require protein precipitation to extract histamine and other biogenic amines from foods, especially fish products. Several extraction solvents were used: hydrochloric acid (Innocente *et al*., 2007), methanol (Zarei *et al*., 2009; AOAC, 2012), trichloroacetic acid (TCA) (Sagratini *et al*., 2012), and perchloric acid (Jančí *et al*., 2017). Among these solvents, TCA is widely used in analytical laboratories with a high recovery yield (Zarei *et al*., 2014; Felisiak *et al*., 2019). However, this technique has major disadvantages in that TCA is highly corrosive and should be handled with care.

The salting-out technique is an operation that consists of adding a high concentration of salt to induce protein precipitation. It increases the difference in polarity between the aqueous and organic phases, which allows the passage of soluble molecules from the organic phase to the aqueous solution (Tu *et al*., 2018; Sazali *et al*., 2019). Salts such as ammonium sulfate and sodium chloride (NaCl) were often used as essential releasers (Grozdanic *et al*., 2014; Sparidans *et al*., 2016). Various natural substances, such as heavy metals, drugs, and ribosomes, have been extracted by the salting-out method (Majors, 2009; Fu *et al*., 2015; Li *et al*., 2016). Our study aims to develop a simple and inexpensive process for the extraction of histamine from fish flesh based on the principle of salting out using NaCl. The effects of physico-chemical parameters such as NaCl concentration, pH, extraction time, and temperature were studied. The recovery rates recorded using NaCl were compared to those obtained with TCA.

Materials and Methods

Fish samples and reagents

This work was carried out on commercial samples of canned tuna in brine spiked with histamine from the food analysis performance assessment scheme (FAPAS) for the Central Science Laboratory Proficiency Testing Group (England). For two samples of different histamine concentrations (FAPAS 1 and FAPAS 2 containing, respectively, 30.7 and 220 mg/kg), we used a total of five cans of 145 g each. These products are certified as standards and are used in analytical performance tests (Rachidi *et al*., 2011).

Fish products come from the local market (Tan-Tan City, southern Morocco). 1.5 kg of flesh from each species, including fresh anchovies and mackerel, canned sardines and tuna, marinated anchovies, and fishmeal, were considered in this study.

TCA, NaCl, ortho-phthaldialdehyde, and histamine dihydrochloride were purchased from Sigma Aldrich (Schnelldorf, Germany). Hydrochloric acid, sodium acetate, and methanol were purchased from Fluka (Buchs, Germany), and sodium hydroxide from Titrisol (Germany).

Extraction of histamine

Histamine extraction was carried out using NaCl and TCA on the two FAPAS products and different fish samples. 5 g of each sample was ground in the presence of 45 mL of extraction solution using a mixer (Waring Laboratory Science™ LB20EG, United States) for 3 minutes. The ground material was recovered using standard pleated filters with a diameter of 150 mm (FiltraTech, Saint-Jean-de-Braye, France) and then stored at 30°C until analysis. The concentrations of NaCl used varied from 0.06 mol/L (0.35%, w/w) up to 7.06 mol/L (41%, w/w), which represents the saturation. The concentration of TCA was 10% (w/w). The extraction temperatures were 4, 20, and 40°C, and the extraction time varied from 1 to 5 minutes. During the extraction, the variation of the pH of the NaCl solution as a function of time was measured using a Hannah Instrument pH meter (Hanna Instrument, Ronchi Di Villafranca Padovana, Italy).

Histamine assay

The purification of histamine was performed according to the method of Lerke and Bell (1976). Histamine was fixed on a column filled with amberlite resin and eluted with 0.2 N hydrochloric acid. The assay was carried out after condensation of the histamine with ortho-phthalaldehyde (1%). The calibration of the fluorimeter (Turner Designs Trilogy 10-AU-904, Turner Designs, San Jose, CA, United States) was achieved at the respective emission and excitation wavelengths of 360 and 450 nm. The yield of histamine extraction was calculated according to the following relationship (Equation 1):

$$Yield\ (\%) = \frac{Histamine\ recovered}{Histamine\ in\ the\ sample} \quad \text{[Eq. 1]}$$

Statistic study

The extraction tests were carried out in triplicate (n=3). The results of the histamine analysis are expressed as mean values ± standard deviation. The level of significance of the differences was determined using Fisher's test (analysis of variance, STATISTICA 6 software, Informer Technologies, Los Angeles, CA, USA). The precision of the analysis was calculated for each standard concentration using the following formulas (Equations 2 and 3):

Repeatability coefficient of variation:

$$\text{Repeatability coefficient of variation: } CVr = \frac{Sr}{\bar{a}} \quad \text{[Eq. 2]}$$

Reproducibility coefficient of variation:

$$\text{Reproducibility coefficient of variation: } CVR = \frac{SR}{\bar{a}} \quad \text{[Eq. 3]}$$

S_r is the repeatability standard deviation, S_R is the reproducibility deviation and ā is the mean of the sample considered.

Results and Discussion

Effect of sodium chloride concentration

Figure 1 shows that the histamine extraction efficiency increases with the concentration of NaCl. The maximum values were obtained with saturating solutions (7.06 mol/L). The histamine recovery rate of FAPAS samples (1 and 2) for an extraction time of 2 minutes at room temperature was 65% and 67%, respectively. Previous works reported that the use of NaCl in different solvents, such as perchloric acid and n-butanol, would increase the yield of histamine extraction (Patange *et al*., 2005; Ramos *et al*., 2014). Indeed, the use of high salt concentrations has the power to neutralize certain ionic charges and increase the solubilized fractions of the molecules to be extracted (Endo *et al*., 2012; Dai *et al*., 2014; Sazali *et al*., 2019). Our results showed that the use of NaCl alone at saturating concentrations makes it possible to have good yields (Vogel, 1996). These results are explained by the effect of salting out, which causes the precipitation of proteins and the solubilization of histamine.

Effect of time

Yields obtained as a function of the extraction time are presented in Figure 2. The maximum yield was recorded from minute 4 (76±2.51% and 79±3.21%, respectively, for FAPAS 1 and FAPAS 2). The statistical analysis revealed no significant variations ($p<0.05$) for the two FAPAS samples, except for minute 1 of extraction. On the other hand, a significant increase in the his-

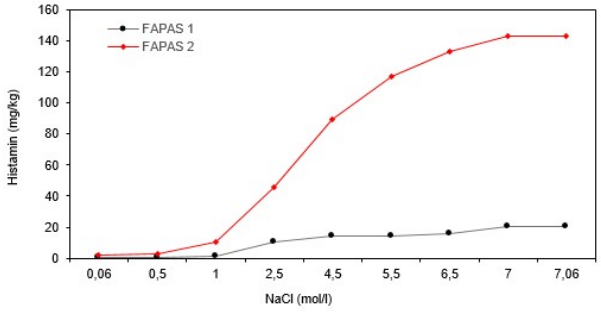

Figure 1. Effect of sodium chloride (NaCl) concentration on histamine extraction in the two food analysis performance assessment scheme (FAPAS) samples at room temperature.

tamine extraction yield of the two FAPAS samples was observed at minute 4 with respect to the extraction time. Novak and Havlíček (2016) reported that for the crude purification of proteins from solution, one of the most used techniques is still precipitation through the addition of salts, specifically ammonium sulfate, and the duration of the extraction improves the extraction yield. This is explained by the interactions of water molecules with proteins, amino acids, and salt ions. Indeed, the reduction in the solvated surface and the increase in protein-protein interactions would foster their precipitation (Tang and Weng, 2013; Hyde *et al*., 2017; Tu *et al*., 2018).

A high concentration of NaCl makes the pH of the solution alkaline. Solubility and extraction yields are generally better at an alkaline pH than at an acidic pH. Indeed, a very alkaline pH can lead to the dissociation and disaggregation of proteins (Fu *et al*., 2017). Adding the fish sample leads to a decrease in the pH of the alkaline NaCl solution (Figure 2). During the extraction of histamine from the saturated solution of NaCl (41%), the pH decreased from 8 at minute 1 to stabilize at 5.2 starting from minute 4 (Figure 2). This significant decrease in pH could be explained by the release of acidic components (like hydrogen ions, amino acids, lactic acid, and acetic acid) from fish flesh (Jiang *et al*., 2010; Tomé *et al*., 2013). It should be noted that the time required for pH stabilization was consistent with the time required to have a high extraction yield. Indeed, the acidity of the medium favors the precipitation of proteins and, consequently, the extraction of histamine (Xu *et al*., 2019).

Effect of temperature

The effect of temperature on the histamine extraction yield of FAPAS samples is illustrated in Figure 3. The values obtained with temperatures 4 and 20°C were similar for FAPAS 1 and FAPAS 2 and ranged from 60 to 70% (60±3.5 and 63±2.1%, and 67±2.02 and 70±3.6%, respectively, for FAPAS 1 and FAPAS 2 at temperatures 4 and 20°C). This yield increased significantly at the extraction temperature of 40°C (97±0.64 and 95±2.35%, respectively, for FAPAS 1 and FAPAS 2).

The improvement in yield at 40°C could be attributed to the denaturation of proteins or the melting of fats under the effect of this temperature (Qixing *et al*., 2014). At lower temperatures, lipids form emulsions that prevent or slow down the extraction of different substances (Sadeghi and Jahani, 2012). The use of a temperature of 40°C would cause the melting of the fat and would then make it possible to bypass the stage of the elimination of the lipids before the extraction.

Comparison between extraction with sodium chloride and trichloracetic acid

Table 1 shows the extraction yields obtained with 41% NaCl and 10% TCA. The statistical analysis did not reveal any significant difference in the yield rate between the two extraction methods (99.15 to 100.1% for TCA and 98.65 to 99.74% for NaCl). The coefficient of variation of reproducibility and repeatability did not exceed 1% for the two extraction methods. This variation remains acceptable (<5%) for both methods (ISO, 2019).

Some histidine-rich fish samples were analyzed. Figure 4 shows that the content of histamine extracted with 41% NaCl and 10% TCA solutions was respectively 15.11±1.23 and 17.47±6.6 in fresh anchovies, 62.54±2.31 and 67.12±4.8 mg/kg in canned tuna, 55.27±3.25 and 57.51±6.62 mg/kg in fresh mackerel, 172.98±5.17

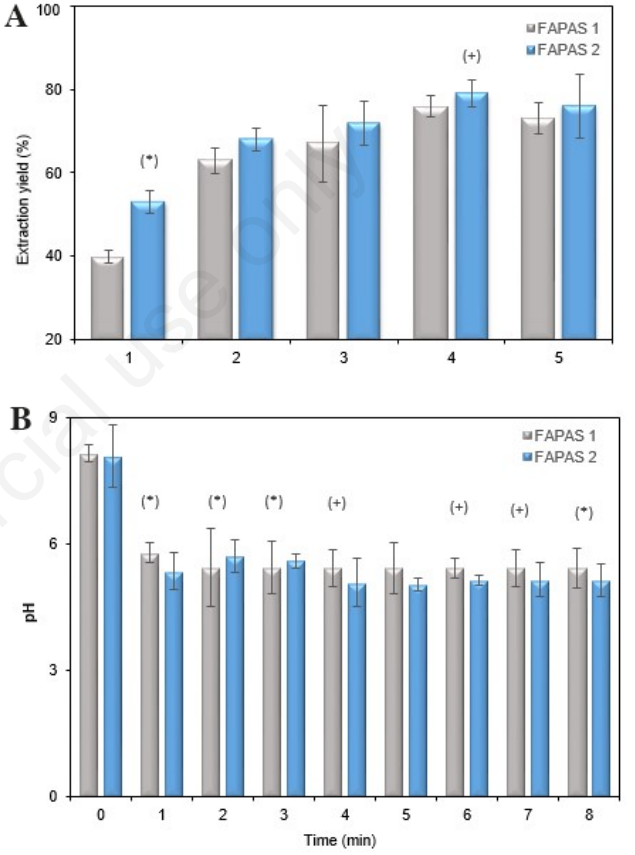

Figure 2. Effect of time on histamine extraction yield (A) and pH (B) of the two food analysis performance assessment scheme (FAPAS) samples using 41% sodium chloride solution at room temperature. Values are means ± standard deviation (n=3). *Significant differences between the two FAPAS samples; +, significant differences: between times of extraction ($p<0.05$).

Table 1. Yields of histamine extraction and coefficients of repeatability and reproducibility obtained with 41% sodium chloride and 10% trichloroacetic acid solutions.

Standard concentration (mg/kg)	NaCl (41%)			TCA (10%)		
	Yield (%)	CV_r (%)	CV_R (%)	Yield (%)	CV_r (%)	CV_R (%)
70	98.65±1.02	1.42	0.53	99.15±0.42	0.48	0.46
120	99.74±0.15	0.6	0.67	100.02±0.22	0.96	0.43
180	99.45±0.24	0.64	0.26	100.1±0.32	0.37	0.52

NaCl, sodium chloride; TCA, trichloroacetic acid; CV_r, coefficient of repeatability; CV_R, coefficient of reproducibility.

and 180.15±3.64 mg/kg in fishmeal, 50.16±4.53 and 50.64±7.21 mg/kg in canned sardines, and 33.2±2.4 and 37.73±5.5 mg/kg in marinated anchovies. The results show a similarity in the evolution of the extraction yields obtained by the two extraction solutions, which makes it possible to use 41% NaCl as an extraction solution in the same way as 10% TCA. Consequently, the extraction efficiency of histamine does not appear to be induced by the type of fish flesh (fresh, canned, marinated...). In the other part, due to the different partition coefficients and solubilities of biogenic amines in extraction media, it appears that the extraction efficiency using the salting-out approach is validated for histamine and may not be suitable for other types of amines.

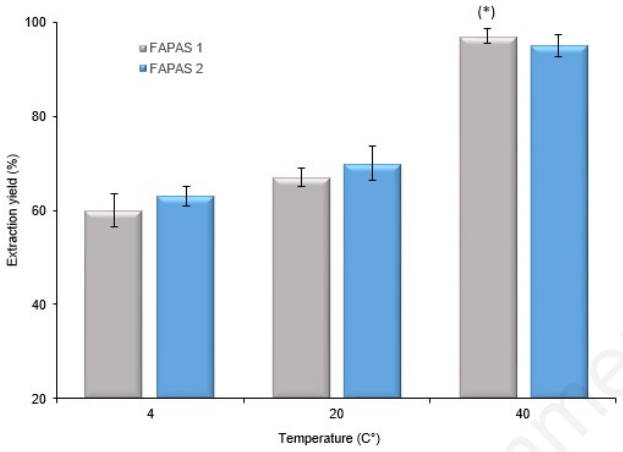

Figure 3. Effect of temperature on the extraction yield of histamine from the two food analysis performance assessment scheme (FAPAS) samples using 41% sodium chloride solution for 4 minutes. Values are means ± standard deviation from triplicate measurements (n=3). *Significantly different from FAPAS 2 ($p<0.05$).

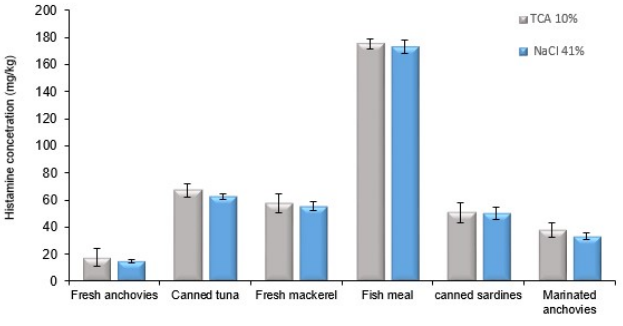

Figure 4. Histamine contents obtained from different fish products using 10% trichloroacetic acid and 41% sodium chloride extraction solutions. Values are means ± standard deviations from triplicate measurements (n=3). NaCl, sodium chloride; TCA, trichloroacetic acid.

Conclusions

This study reports a new method for the extraction of histamine from fish flesh. It uses a high concentration of NaCl (41%), which makes it possible to obtain a yield similar to that obtained with TCA (10%). It has several advantages: speed, reproducibility, ease of implementation, and lower cost than other solvents. In addition, the use of NaCl for extraction minimizes the risks associated with the use of toxic chemicals such as TCA, whether for users in the food industry or for the environment. The use of NaCl can therefore be generalized to replace chemical solvents such as TCA.

References

AOAC, 2012. Method 977.13. Histamine in seafood: fluorometric method. In: Association of Official Analytical Chemists 16th ed. AOAC International, Gaithersburg, USA.

Biji KB, Ravishankar CN, Venkateswarlu R, Mohan CO, Srinivasa TK, 2016. Biogenic amines in seafood: a review. J Food Sci Technol 53:2210-8.

Bilge B, Hüseyin G, 2015. Determination of biogenic amines in fish products. Food Sci Biotechnol 24:1907-13.

Dai JY, Sun YQ, Xiu ZL, 2014. Separation of bio-based chemicals from fermentation broths by salting-out extraction. Eng Life Sci 14:108-17

Endo S, Pfennigsdorff A, Goss KU, 2012. Salting-out effect in aqueous NaCl solutions: trends with size and polarity of solute molecules. Environ Sci Technol 46:1496-503.

FDA, 2020. Fish and fishery products - hazards and controls guidance 4th ed. Food and Drug Administration, Gainesville, United States, pp 113-52.

Felisiak K, Szymczak M, Kołakowski E, 2019. Identification of non-protein nitrogen compounds separated by CZE without derivatization from TCA extract from salted herring meat. J Food Compos Anal 77:108-14.

Fu H, Sun Y, Teng H, Zhang D, Xiu Z, 2015. Salting-out extraction of carboxylic acids. Sep Purif Technol 139:36-42.

Fu H, Wang X, Sun Y, Yan L, Shen J, Wang J, Shang Y, Xiu Z, 2017. Effects of salting-out and salting-out extraction on the separation of butyric acid. Sep Purif Technol 180:44-50.

Grozdanic ND, Calado MS, Kijercanin M, Serbanovic SP, 2014. Aqueous nicotine solutions: pH-measurements and salting-out effects – analysis of the effective Gibbs energies of hydration and ionic strengths of the solutions. J Serb Chem Soc 79:829-42.

Hyde AM, Zultanski SL, Waldman JH, Zhong YL, Shevlin M, Peng F, 2017. General principles and strategies for salting-out informed by the hofmeister series. Org Process Res Dev 21:1355-70.

Innocente N, Biasutti M, Padovese M, Moret S, 2007. Determination of biogenic amines in cheese using HPLC technique and direct derivatization of acid extract. Food Chem 101:1285-89.

ISO, 2019. Accuracy (trueness and precision) of measurement results and methods. Part 2: basic methods for determining the repeatability and reproducibility of a standard measurement method. ISO Norm 5725-2:2019. International Standardization Organization ed., Geneva, Switzerland.

Janči T, Valinger D, Gajdoš KJ, Mikac L, Vidaček S, Ivanda M, 2017. Determination of histamine in fish by surface enhanced raman spectroscopy using silver colloid SERS substrates. Food

Chem 224:48-54.
Jiang J, Xiong YL, Chen J, 2010. pH shifting alters solubility characteristics and thermal stability of soy protein isolate and its globulin fractions in different pH, salt concentration, and temperature conditions. J Agric Food Chem 58:8035-42.
Lerke PA, Bell LD, 1976. A rapid fluorometric method for the determination of histamine in canned tuna fish. J Food Sci 41:1282-84.
Li FF, Li Q, Wu SG, Tan ZJ, 2016. Salting-out extraction of allicin from garlic (Allium sativum L.) based on ethanol/ammonium sulfate in laboratory and pilot scale. Food Chem 217:91-7.
Majors RE, 2009. Salting-out liquid-liquid extraction (SALLE). LCGC N Am J 27:526-33.
Novak P, Havlíček V, 2016. Protein extraction and precipitation. In: Ciborowski P, Silberring J (ed). Proteomic Profiling and analytical chemistry: the crossroads. Elsevier, New York, USA, pp 52-62.
Patange SB, Mukundan MK, Ashok KK, 2005. A simple and rapid method for colorimetric determination of histamine in fish flesh. Food Control 16:465-72.
Prester L, 2011. Biogenic amines in fish products and shellfish. Food Addit Contam Part A Chem Anal Control Expo Risk Assess 28:1547-60._
Qixing J, Zhengran M, Shuoshuo W, Yanshun X, Fengyu T, Xueqin X, Wenshui X, 2014. Effect of temperature on protein compositional changes of big head carp (Aristichthys nobilis) muscle and exudates. Food Sci Technol Res 20:655-61.
Rachidi A, Oualit N, Touti A, Duflos G, Nôel L, Caillaud P, 2011. Proficiency test scheme for histamine analysis in fishery products in Morocco using the fluorometric method and comparison of results with those obtained with the HPLC. Int J Metrol Qual Eng 2:91-100.
Ramos RM, Valente IM, Rodrigues JA, 2014. Analysis of biogenic amines in wines by salting-out assisted liquid-liquid extraction and high-performance liquid chromatography with fluorimetric detection. Talanta 124:146-51.
Sadeghi R, Jahani F, 2012. Salting-in and salting-out of water-soluble polymers in aqueous salt solutions. J Phys Chem B 116:5234-41.
Sagratini G, Fernández FM, De Berardinis F, Font G, Vittori G, Mañes J, 2012. Simultaneous determination of eight underivatized biogenic amines in fish by solid phase extraction and liquid chromatography–tandem mass spectrometry. Food Chem 132:537-43.
Sazali NH, Alshishani A, Saad B, Chew KY, Chong MM, Miskam M, 2019. Salting-out assisted liquid-liquid extraction coupled with high-performance liquid chromatography for the determination of vitamin D3 in milk samples. Roy Soc Open Sci 6:190952.
Sparidans RW, Hoppe VS, Rood JJM, Schinkel AH, Schellens JHM, Beijnen JH, 2016. Liquid chromatography-tandem mass spectrometric assay for the tyrosine kinase inhibitor afatinib in mouse plasma using salting-out liquid-liquid extraction. J Chromatogr B Analyt Technol Biomed Life Sci 1012-1013:118-23.
Tang YQ, Weng N, 2013. Salting-out assisted liquid-liquid extraction for bioanalysis. Bioanalysis 5:1583-98.
Tomé LI, Pinho S, Jorge M, Gomes, Coutinho J, 2013. Salting-in with a salting-out agent: explaining the cation specific effects on the aqueous solubility of amino acids. J Phys Chem B 117:6116-28.
Tu X, Sun F, Wu S, Liu W, Gao Z, Huang S, Chen W, 2018. Comparison of salting-out and sugaring-out liquid-liquid extraction methods for the partition of 10 -hydroxy-2-decenoic acid in royal jelly and their co-extracted protein content. J Chromatogr B Analyt Technol Biomed Life Sci 1073:90-5.
Vogel AI, Tatchell AR, Furnis BS, Hannaford AJ, Smith PWG, 1996. vogel's textbook of practical organic chemistry. Longman Scientific & Technical, London, UK.
Xu Q, Hong H, Yu W, Jiang X, Yan X, Wu J, 2019. Sodium chloride suppresses the bitterness of protein hydrolysates by decreasing hydrophobic interactions. J Food Sci 84:86-91.
Zarei M, Fazlara A, Najafzadeh H, Zolfaghar K F, 2014. Efficiency of different extraction solvents on recovery of histamine from fresh, frozen and canned fish. J Food Qual Haz Control 1:72-6.
Zarei M, Hosseini A, Shekarforoush S, 2009. Generation of bioluminescent Morganella morganii strain and its potential usage in determination of growth limits and histamine production. J Food Saf 29:190-206.