JOURNAL OF FOOD QUALITY & HAZARDS CONTROL
SHAHID SADOUGHI UNIVERSITY OF MEDICAL SCIENCES & HEALTH SERVICES

Journal of Food Quality and Hazards Control 1 (2014) 72-76

Efficiency of Different Extraction Solvents on Recovery of Histamine from Fresh, Frozen and Canned Fish

M. Zarei[1*], A. Fazlara[1], H. Najafzadeh[2], F. Zolfaghar Karahroodi[3]

1. Department of Food Hygiene, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
2. Department of Basic Sciences, Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran
3. Faculty of Veterinary Medicine, Shahid Chamran University of Ahvaz, Ahvaz, Iran

Article type
Original article

Keywords
Histamine
Solvents
Fish Products
Analysis

Received: 30 Mar 2014
Revised: 27 Apr 2014
Accepted: 23 June 2014

Abstract

Background: Histamine is a food-borne chemical hazard which causes scombroid poisoning. The efficiency of histamine recovery from fish is greatly influenced by selection of an appropriate extraction solvent. Hence, in this study the efficiency of different extraction solvents on recovery of histamine from fresh, frozen and canned fish was evaluated.

Methods: Fresh, frozen and canned rainbow trout samples were homogenized in six different extraction solvents including 0.1 N hydrochloric acid, 5% trichloroacetic acid, 75% methanol, 75% ethanol, 75% methanol-0.4 N hydrochloric acid and 75% ethanol-0.4 N hydrochloric acid. Samples were evaluated for recovery of known quantities of histamine added to fish tissue prior to extraction. The percent values of the recoveries were compared using ANOVA (SPSS 16.0).

Results: Findings of this experiment indicated that different extraction solvents provided different overall mean recoveries for histamine in fish tissue. Using a combination of acid and organic solvents provided a more efficient extraction solvent for histamine from fish tissue than acid or organic solvents alone. In addition, among the solvents used in this study, 75% ethanol-0.4 N HCl resulted in a more complete recovery of added histamine from fresh, frozen and canned fish tissue.

Conclusion: In order to obtain a more accurate measure of histamine in fish tissue, 75% ethanol-0.4 N hydrochloric acid appears to be a good choice for extraction.

Introduction

Histamine is a major chemical hazard of seafood products. It is the causative agent of histamine or scombroid poisoning and is formed by time/temperature abuse of fish muscle (FDA, 2001). Histamine poisoning is usually a mild illness and lasts a few hours, but may continue for several days. The most common symptoms of histamine poisoning include rash, urticaria, nausea, vomiting, diarrhea, headache, palpitations, flushing, tingling and itching of the skin (Lehane and Olley, 2000). Severity of the symptoms can vary considerably with the individual sensitivity to histamine and the amount of ingested histamine. Scombroid fish such as tuna and several species of non scombroid fish contain high levels of free histidine in their muscles which can convert to histamine through the proliferation of bacteria that synthesize histidine decarboxylase. If the fish are subjected to short-time/high-temperature exposure, organoleptic assessment is not able to indicate a safety problem; because these conditions do not cause strong odors of decomposition (Kim et al., 2001). Furthermore, histamine is heat resistant and cannot be eliminated by cooking, freezing, or processing such as canning and smoking (FAO, 2001; Lehane and Olley, 2000).

*Corresponding author
E-mail: zarei@scu.ac.ir

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Therefore, with respect to health and food safety, it is important to accurately monitor histamine levels in seafood. The U.S. Food and Drug Administration established guidelines specifying 500 mg histamine/kg fish as the toxicity level and 50 mg histamine/kg fish as the hazard action level. Seafood products containing above the hazard action level of histamine may not be used for human consumption and must be recalled (FDA, 2002).

A number of chromatographic methods have been proposed for the quantitative determination of histamine in seafood including thin-layer chromatography, ion-exchange chromatography, gas chromatography and high performance liquid chromatography (Lehane and Olley, 2000; Moret and Conte, 1996). All the cited methods involve two main steps, i.e. histamine extraction from the matrix including purification of the raw extract and determination of histamine. The first phase is the most critical in term of obtaining an adequate recovery for histamine. The efficiency of histamine recovery from fish is greatly influenced by the selection of an appropriate extraction solvent (Custodio et al., 2007; Moret and Conte, 1996; Richard et al., 2008). Currently, the official methods of the Association of Official Analytical Chemists (AOAC) uses 75% methanol–water as the extraction solvent in canned tuna to determine histamine; however, this extraction solvent may not be optimal for fresh or frozen fish (AOAC, 2000).

To the best of our knowledge, only a few studies have focused on the impact of extraction solvent type on histamine analysis in fish tissue. Hence, the objective of the present study was to assess the efficiency of different extraction solvents on recovery of histamine from representative fresh, frozen and canned fish.

Materials and methods

Sample preparation

Fresh rainbow trout (*Oncorhynchus mykiss*), varying from 350 g to 400 g in weight, were purchased from an Iranian public market and transported in crushed ice to the laboratory. After being gutted and washed, they were randomly assigned into three treatment groups including fresh, frozen and canned groups. Nine fresh samples were used for the analysis directly. To prepare frozen samples, nine fish were individually put in plastic bags and stored at -18 ℃ for 15 days. To provide canned samples, nine fish were individually put in glass bottles and autoclaved at 121 ℃ for 15 min and then stored at room temperature until analysis step.

Reagents

All reagents were of analytical reagent grade. Dowex 1-X8 resin, O-phthaldialdehyde (OPT) and histamine dihydrochloride were purchased from Sigma (Sigma, St. Louis, MO). Methanol, ethanol, hydrochloric acid (HCl) and trichloroacetic acid (TCA) were purchased from Merck (Merck, Darmstadt, Germany).

All the glassware was washed with 0.1 N HCl and then rinsed with deionized water before use. The standard stock solutions used for calibration were produced by dissolving 169.1 mg histamine dihydrochloride in 100 ml 0.1 N HCl. The standard working solutions and the spiking solutions were freshly prepared by diluting an aliquot of the stock solution using 0.1 N HCl.

Histamine extraction

Extractions were performed with each of six solvents: 0.1 N HCl, 5% TCA, 75% methanol, 75% ethanol, 75% methanol-0.4 N HCl and 75% ethanol-0.4 N HCl. Fresh, frozen and canned fish samples were filleted and ground in a food processor. Each sample (10 g) was homogenized in 70 ml extraction solvent and incubated at 60 ℃ for 15 min. Samples were then cooled at room temperature and contents were transferred to 100 ml volumetric flasks. The volumetric flasks were diluted to volume with the appropriate extraction solvent and mixed by inverting. The mixture was filtered through folded filter paper. Extracts were stored in refrigerator (4–5 °C) until analysis (AOAC, 2000).

For spiked sample extracts, 1 ml spiking solutions (300 and 600 µg/ml) was added to 10 g fish sample and homogenized. Then, 70 ml of extraction solvent was added to the sample, incubated at 60 °C for 15 min and followed as explained above. The extracts were stored in a refrigerator (4–5 °C) for future analysis.

Ion exchange chromatography

One ml of the extracts was subjected to ion exchange chromatography on an 80×5 mm column of Dowex 1-X8 resin, which was converted to hydroxide form by 2 N NaOH. The column was washed with 35 ml of deionized water. The eluate was collected in a 50 ml volumetric flask containing 5 ml 1 N HCl and the volume was adjusted to 50 ml with deionized water (AOAC, 2000).

Apparatus for histamine analysis

A synergy HT multimode microplate reader (BioTek Instruments) equipped with Gen 5 software was used to determine the fluorescence intensity at excitation wavelength of 350 nm and emission wavelength of 444 nm.

Preparation of the fluorescent histamine derivative

Histamine standards (0.1, 0.2 and 0.3 µg/ml) and the column eluates were derived with OPT. Five ml of the column eluates or the standards and 10 ml of 0.1 N HCl were added into a 50 ml flask, followed by the addition of 3 ml of 1 N NaOH and 1 ml of 0.1% (w/v) OPT solution, consecutively. The mixture was shaken thoroughly and after

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exactly 4 min, 3 ml of 3.57 N phosphoric acid was added and mixed immediately. A blank was prepared by substituting 5 ml 0.1 N HCl for histamine solution. Fluorescence intensity was measured during 1.5 h (AOAC, 2000).

Statistical analysis

Results were analysed using ANOVA (SPSS 16.0). The significance levels are expressed at a 95% confidence level (p<0.05) throughout.

Results

Among six different solvents investigated in this study, 0.1 N HCl was not a good choice for the extraction of histamine from fresh, frozen and canned fish samples. Difficulties related to sample turbidity after homogenization and filtration as well as blocking the chromatographic column with the turbid extract were observed. However, 5% TCA showed better results and extracted histamine from fresh, frozen and canned fish samples, but its recovery value was not perfect. As shown in Table 1, Table 2 and Table 3, almost the same results were observed for 75% methanol and 75% ethanol. These organic solvents showed low levels of histamine recovery in fresh, frozen and canned fish samples. On the other hand, using a combination of acid and organic solvents resulted in a higher level of recovery.

In fresh fish samples, 75% methanol-0.4 N HCl and 75% ethanol-0.4 N HCl showed significantly higher levels (p<0.05) of histamine recovery, compared to acids or organic solvents. In addition, the level of histamine recovery in the presence of 75% ethanol-0.4 N HCl was significantly higher (p<0.05) than 75% methanol-0.4 N HCl (Table 1). Almost the same pattern for recovery levels was observed in frozen and canned fish samples and 75% ethanol-0.4 N HCl was the best solvent for the extraction of histamine (Table 2 and Table 3).

Table 1: Recovery values of added histamine to fresh fish using different extraction solvents

Solvent	Spiked with 30 mg histamine/kg fish (%±SD)	Spiked with 60 mg histamine/kg fish (%±SD)
HCl 0.1 N	N.Q.*	N.Q.
TCA 5%	57±23.5 b**	52.8±14 a
Methanol 75%	60.7±17.3 b	56.3±17.3 a
Ethanol 75%	46±14 a	56.3±7.8 a
Methanol 75%-HCl 0.4 N	78±15 c	60.6±4.3 a
Ethanol 75%-HCl 0.4 N	112±17 d	78±19.8 b

*N.Q.: not quantifiable
** Different letters (a–d) within columns are significantly different at p<0.05

Table 2: Recovery values of added histamine to frozen fish using different extraction solvents

Solvent	Spiked with 30 mg histamine/kg fish (%±SD)	Spiked with 60 mg histamine/kg fish (%±SD)
HCl 0.1 N	N.Q.*	N.Q.
TCA 5%	75.6±2.5 b**	54.4±7.9 a
Methanol 75%	69.4±8.6 a	71.8±6.8 b
Ethanol 75%	83.4±20.6 b	86.7±4.3 c
Methanol 75%-HCl 0.4 N	79.7±7.9 b	82.4±4.3 c
Ethanol 75%-HCl 0.4 N	95.4±8.7 c	112.7±8.6 d

*N.Q.: not quantifiable
** Different letters (a–d) within columns are significantly different at p<0.05

Table 3: Recovery values of added histamine to canned fish using different extraction solvents

Solvent	Spiked with 30 mg histamine/kg fish (%±SD)	Spiked with 60 mg histamine/kg fish (%±SD)
HCl 0.1 N	N.Q.*	N.Q.
TCA 5%	60.7±17.3 b**	60.9±11.3 b
Methanol 75%	43.3±8.6 a	63.3±11.4 b
Ethanol 75%	60.7±22.9 b	52±13 a
Methanol 75%-HCl 0.4 N	69.4±8.7 c	78±19.8 c
Ethanol 75%-HCl 0.4 N	78±15 d	99.7±15.5 d

*N.Q.: not quantifiable
** Different letters (a–d) within columns are significantly different at p<0.05

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Discussion

The extraction of biogenic amines from diverse food products can be carried out with water, at room temperature (Ingles et al., 1985) or higher temperatures (Voigt et al., 1974) so that only free amines are extracted, or in an acid medium, with hydrochloric acid (Cinquina et al., 2004; Gennaro et al., 2003; Innocente et al., 2007; Martuscelli et al., 2005), perchloric acid (Minocha and Long, 2004; Novella-Rodriguez et al., 2000; Pinho et al., 2001) or trichloroacetic acid (Chang et al., 2008; Lapa-Guimaraes and Pickova, 2004; Saaid et al., 2009; Zarei et al., 2011), so that amines linked to other matrix components can be extracted.

In the present study, two acids including 0.1 N HCl and 5% TCA were used to extract histamine from fish samples. Due to the inability of HCl to precipitate proteins of fish samples and turbidity of the resulted extract, this acid did not exhibit good results in this study. On the contrary, Custodio et al. (2007) reported that HCl extracted the highest level of histamine from cheese compared to other solvents. On the other hand, in our study, 5% TCA was able to precipitate proteins in fish homogenates and prepare a clear extract for ion exchange chromatography; however, its recovery value was not perfect.

Several organic solvents, such as methanol (AOAC, 2000; Zarei et al., 2009), and ethanol (Sato et al., 1970) have also been used for the extraction of biogenic amines. Moreover, a combination of organic solvents and acid can be used for this purpose such as dichloromethane-$HClO_4$ (Takeba et al., 1990).

Results of the present investigation indicated that different extraction solvents provided different overall mean recoveries of histamine in fish tissue. It was found that 5% TCA, 75% methanol and 75% ethanol showed low levels of histamine recovery in fresh fish samples, while a combination of acid and organic solvents resulted in a higher level of recovery. In this case, the combination of 75% ethanol-0.4 N HCl showed the highest level of histamine recovery in fresh, frozen and canned fish samples. Methanol which was considered by AOAC (2000) as an ideal solvent for histamine extraction from canned fish, resulted in significantly (p<0.05) lower recovery than 75% methanol-0.4 N HCl. Acidification of the extraction solvent allowed a more complete recovery of added histamine from the fish tissue matrix. This may be the result of improved release of the histamine under acidic conditions. However, the extraction efficiency of 75% methanol-0.4 N HCl was lower than 75% ethanol-0.4 N HCl. This may be due to the higher ability of ethanol to extract histamine in fish homogenates compared to methanol. According to Custodio et al. (2007), ethanol extracted higher level of histamine from cheese compared to methanol. They reported that methanol did not provide good recovery of histamine for cheese samples.

Richard et al. (2008) reported that methanol extraction of biogenic amines resulted in significantly (p<0.05) lower recovery than methanol–HCl extraction, where recoveries increased, respectively, for putrescine from 44 to 100% (flounder and scup) and from 42 to 119% (butterfish and mackerel A); for cadaverine from 47 to 106% (flounder and scup) and from 49 to 113% (butterfish and mackerel), and for histamine from 54 to 89% (mackerel). However, it appears that the extraction efficiency of methanol–HCl may not be appropriate for different types of foods such as cheese. Custodio et al. (2007) observed lower recovery of cadaverine and histamine extracted from grated Parmesan cheese when methanol–HCl was used. Consequently, the extraction efficiency of biogenic amines appears to be influenced by the type of food. Moreover, each solvent provides different recovery values for the various groups of amines, probably because of the different partition coefficients and solubilities of the amines in the extraction media (Custodio et al., 2007; Moret and Conte, 1996).

Custodio et al. (2007) showed that, for the extraction of different biogenic amines from cheese, the polyamines, spermine and spermidine were better recovered by HCl; agmatine was better extracted by borate buffer or HCl; histamine was better extracted by HCl or by ethanol; the aliphatic amines, putrescine and cadaverine were more efficiently extracted by borate buffer and HCl; whereas the aromatic amines, tyramine and phenylethylamine and the indolamines, tryptamine and serotonin were better recovered with organic solvents.

Conclusion

According to the results of the present study, using a combination of acid and organic solvents provided a more efficient extraction solvent for histamine from fish tissue than acid or organic solvents alone. In addition, among the solvents used in this study, 75% ethanol-0.4 N HCl resulted in a more complete recovery of added histamine from fresh, frozen and canned fish tissue.

Conflicts of interest

The authors declare that they have no conflict of interest in this research.

Acknowledgement

This study was funded by the research grants provided by Shahid Chamran University of Ahvaz, Iran. The authors would like to thank Mrs. P. Esfahani for her kind assistance.

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