

Biogenic amine contents of commercially processed traditional fish products originating from European countries and Turkey

Sevim Köse · Serkan Koral · Bekir Tufan ·
Matevž Pompe · Andrej Scavničar · Drago Koçar

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Abstract In this study, 65 traditional fish samples, 35 of which originated from 11 European countries and 30 from Turkey were analysed for biogenic amines, water activity (a_w), pH, %salt, %water phase salt, moisture contents and microbial counts in terms of food safety. In total, eleven samples contained histamine values between 70 and 1,544 mg/kg, which are over FDA permitted level of 50 mg/kg, four of which also exceeded EU and Turkish permitted limit of either 100 or 200 mg/kg. The highest histamine value was attributed to fish paste at 1,544 mg/kg and among other biogenic amines, the highest value was observed for cadaverine at 1,862 mg/kg represented by smoked bonito. Putrescine levels in most of the fermented samples were found to be high between 141 and 836 mg/kg. Some of the other highest levels of biogenic amines were phenylethylamine, tyramine and tryptamine at 219, 783 and 517 mg/kg, respectively. This study shows that although most products of traditional fish-processing types from these countries are safe for biogenic amine development, some are still lacking effective preventive actions. This situation suggests the awareness on seafood safety for such processing types.

Keywords Traditional fish · Biogenic amines · Histamine · Food safety · Fish paste

S. Köse (✉) · S. Koral · B. Tufan
Department of Fisheries Technology Engineering,
Faculty of Marine Sciences, Karadeniz Technical University,
61530 Trabzon, Turkey
e-mail: sevimkose@yahoo.com; kosesevim@gmail.com

M. Pompe · A. Scavničar · D. Koçar
Department of Analytical Chemistry, Faculty of Chemistry
and Chemical Technology, University of Ljubljana,
1000 Ljubljana, Slovenia

Introduction

Traditionally processed fish products are reported to carry a high potential risk for human health mainly caused by halophilic pathogenic bacteria and biogenic amines. Because of their risk for human health and legal criteria for potential health hazards, domestic and international marketing of these products are becoming difficult and limited [1].

Biogenic amines are mainly formed in foods through decarboxylation of specific free amino acids by exogenous decarboxylase released by microbial species associated with food and by transamination of aldehyde and ketones. The chemical structure of these amines can either be aliphatic, aromatic or heterocyclic. Various authors had classified cadaverine, putrescine, spermine and spermidine as polyamines [2]. Certain biogenic amines such as histamine, cadaverine, putrescine and tyramine are of importance because of their risk for food intoxication and also they serve as chemical indicators of fish spoilage [1, 3].

Histamine is one of the main concerns in fishery products formed by microbial decarboxylation of histidine as a result of time/temperature abuse in certain fish species. Many different bacterial species have been reported to have histidine decarboxylase that has the ability to produce histamine [1]. Although histamine-poisoning cases/outbreaks have been reported worldwide originating from traditional fish products [3, 4], many incidents are claimed to be unreported [1, 5]. Development of biogenic amines in traditional fish products occurs due to poor handling of the raw materials, improper salt maturation or fermenting stage and storage conditions [1].

The US Food and Drug Administration (FDA) [6] has set 50 mg/kg histamine limit for most fish products, while

less than 500 mg/kg is accepted for fish sauce [7]. European Community (EC) regulations have fixed a maximum of 100 mg/kg as the average value of nine samples of fresh or canned fish and 200 mg/kg for fermented fish or other enzymatically ripened products [8]. Turkish authorities follow-up the 100 mg/kg limit only for fresh and frozen unprocessed fish products. The acceptable limit for histamine in all processed fish products is given as 200 mg/kg [9].

Despite similarities, traditional fish products of each region/countries may vary in great extent in terms of processing techniques, product properties such as pH value, a_w , salt content and other ingredients used [1, 10]. This variation may also depend on different producers/brands and can lead to differences in the levels of biogenic amines and other quality and safety parameters of the products [1].

Several experimental trials have demonstrated the development of biogenic amines in various traditional fish products such as marinated anchovy and sardine [11], cold-smoked salmon [12] and fermented fish [13]. Experimental trials were also supported by the studies with commercial traditional fish products that various products contain high levels of biogenic amines, particularly histamine. However, most studies are related to traditional (ethnic) products originating from Africa or East and Southeast Asia [14–17]. On the other hand, limited reports exist on the presence of such amines in commercial traditional fish products of European countries and Turkey. Earlier reports have showed differences in the levels of biogenic amines at different types of traditional fish products within the same country and also different countries. Differences were also found amongst different traditional fish products [5, 14, 18]. Brillantes et al. [13] demonstrated that histamine was formed both in the raw material and during fermentation. Therefore, they speculated that histidine decarboxylase enzymes formed prior to fermentation produced histamine during fermentation. Shakila et al. [19] pointed out that a delay in hot blanching and smoking stage of processing of tuna could contribute to the amine formation because of the presence of potential amine-forming bacteria even if the initial bacteriological quality of tuna is good. Moreover, Gökoğlu [11] showed that low pH might have enhanced histamine formation as in the case of marinated products.

Therefore, this study aims to investigate the biogenic amine content of various commercial traditional fish products originating from Turkey and European countries in order to evaluate the health risk of these processing techniques. Due to the numerous types of traditional fish products from European countries and Turkish origin, only higher risk groups (smoked, marinated, fermented and fish paste) were targeted in this work.

Materials and methods

Materials

Samples

Three main types of traditional fish products, namely fermented including fish paste, marinated and smoked samples were chosen for this study. Products within the same type varied according to commercial brands although some similarities existed. The samples were bought both directly from the producer company (for most fermented fish) and from supermarkets of different countries. They were transported to the laboratory in chilled storage conditions at 4 ± 3 °C except for fermented products from Sweden. The fermented samples from Sweden were shipped to Trabzon, Turkey, at ambient temperature.

Sampling plan

All products were analysed within shelf-life stated by the producer company. After microbial analysis in aseptic conditions, the remaining flesh of the samples were separated from the skins and bones manually, and then they were minced using kitchen food processor (Arçelik, K-1631 P Valso Plus, 2.2 L capacity, Turkey) for 5 min. The minced products were kept at 4 ± 1 °C in a refrigerator (8810NF; Arçelik, İzmit, Turkey) prior to analysis for the rest of the food safety parameters (a_w , pH, moisture and salt contents) and biogenic amines. If the analysis of biogenic amines was delayed, the minced samples for this analysis were frozen and stored in a freezer at -40 ± 2 °C (Sanyo, MDF-U5411, Moriguchi City, Osaka, Japan) and defrosted at the refrigerated temperatures prior to analysis.

Chemicals

All chemicals and solvents used were of analytical and chromatographic grade provided from various companies (Fluka, Switzerland, USA & Germany; Sigma–Aldrich, Switzerland; Merck, Darmstadt, Germany; Carlo Erba, Milano, Italy; VWR Prolabo, Leuven, Belgium; Amresco, OH, USA).

Methods

Microbial analysis

Microbial counts were carried out immediately after the arrival of samples to the laboratory and before the analysis of other parameters. The counts of total viable bacteria were carried out according to Koral and Köse [20]. Total halophilic bacteria were determined using the plate count

Table 1 Method characteristics (*r*, LOD, LOQ) of HPLC method for biogenic amines and %RSD for some traditional fish samples

Amine type	<i>r</i>	LOD	LOQ	%RSD values (n:10 for each sample)			
				Fermented herring	Anchovy paste	Smoked salmon	Marinated anchovy
Triptamine	0.999	1.804	5.46	2.28	1.16	0.31	2.04
Phenylethylamine	0.999	1.509	4.57	3.69	1.85	1.81	2.68
Putrescine	0.999	0.567	1.71	3.24	0.85	0.94	2.64
Cadaverine	0.999	0.666	2.01	2.34	0.63	1.31	3.33
Histamine	0.999	0.848	2.57	2.77	0.77	2.40	1.60
Tyramine	0.999	0.872	2.64	2.91	1.02	1.55	2.46
Spermidine	0.999	0.654	1.98	2.75	2.45	2.58	1.44
Spermine	0.999	0.715	2.16	3.74	2.58	1.69	2.63

RSD relative standard deviation, LOD limit of determination, LOQ limit of quantification

agar containing 8 % NaCl [21]. Total histamine-forming bacteria were determined according to method described by Köse [22] using total histamine-forming bacteria isolation agar. Twenty-five grams of samples was aseptically weighed into a sterile stomacher bag containing 225 mL of sterile physiological saline (0.85 %) and homogenized using a stomacher (Mayo, HG400V, Italy) for 4 min at speed 4. Further decimal dilutions were prepared in physiological saline (0.85 %). The inoculated Petri plates were incubated at 37 °C for 48 h. Microbial counts were done in duplicate, and the mean values were expressed as log cfu/g.

Analysis of biogenic amines

A high-performance liquid chromatography (HPLC) method was used as described in Koral and Köse [22] and Köse et al. [23]. This method involves dansyl derivatization. Shimadzu Prominence LC-20 AT series (Japan) HPLC with autosampler (SIL20AC, Shimadzu, Japan), Diode Array Detector (SPD-M20A, Shimadzu, Japan) and Intertsil column (GL Sciences, ODS-3, 5 µm, 4.6 × 250 mm) were used.

To extract BAs, 10 mL of 0.4 M perchloric acid was added to 5 g sample, and the mixture was homogenized using Ultra-turrax homogenizer (IKA T 25, Digital, Germany) in an ice bath and centrifuged (MPW 350R. MPW Med. Instruments, Warsaw, Poland) at 3,000×*g* at 4 °C for 10 min. The supernatant was collected, and the residue was extracted again with 10 mL of 0.4 M perchloric acid solution. Both supernatants were combined and filtered through Whatman paper (No. 42). The final volume was adjusted to 25 mL with 0.4 M perchloric acid. The analytical procedure using HPLC instrumentation was carried out as described by Koral and Köse [22].

Biogenic amine analysis was carried out in triplicate. Reproducibility of the method was determined by analysing 10 parallels of the same sample according to Massart et al. [24]. Method characteristics and % of relative

standard deviations (%RSD) are given in Table 1. Recovery of each amine for different types of traditional fish products was estimated for each biogenic amine according to Köse et al. [23]. Spiked samples used were as follows: marinated anchovy, smoked salmon, anchovy paste and fermented Baltic herring. Four different spiking amounts (10, 25, 50 and 100 mg/kg) were used to estimate % recovery. The results of % recovery are shown in Table 2.

Other measurements

Moisture content was determined by oven-drying of 5 g of fish muscle at 105 °C until a constant weight was obtained using the method of AOAC [25] (Method 985.14). Results were expressed as g water/100 g muscle. Moisture content was used to calculate WPS % in the samples. Mohr method was used to determine salt content in fish muscle as described in Keskin [26]. WPS is the amount of salt in the product relative to the product moisture and is found using the following calculation [27]. %WPS = [%salt/(%salt + %moisture)] × 100. Water activity was measured using Aqualab water activity meter 3TE (0.100–1.000 ± 0.003, USA) model. The pH measurements were taken with a digital pH meter (Jenco 6230N, CA, USA) by placing the electrode into the homogenized samples in 5 g of fish flesh plus 10 mL of distilled water.

All measurements were taken from the triplicated samples (same lot, different packages or pieces).

Results and discussion

Percentage recovery for different biogenic amines using different types of samples analysed by HPLC method are given in Table 2. A wide range of recovery was observed for different biogenic amines with the effect of sample type and the amount of spike used. The best recoveries usually obtained with 100 mg/kg spiking level were between 90

Table 2 Percentage of recovery for biogenic amines in some traditional fish samples determined by HPLC using four different spike levels

Spike amounts 10, 25, 50 and 100 mg/kg for each amine. *n*: 3 for each spike volume for each type of sample

Amine type	%Recovery			
	Fermented shad	Marinated anchovy	Smoked salmon	Anchovy paste
Triptamine	87.7–108.7	68.9–70.7	75.0–83.7	95.5–103.1
Phenylethylamine	78.8–105.3	84.0–97.0	94.3–97.7	82.4–91.0
Putrescine	83.8–101.8	88.7–100.3	75.1–92.5	94.7–98.2
Cadaverine	79.3–92.8	90.6–96.3	75.6–96.8	80.1–97.5
Histamine	89.0–99.9	77.6–94.8	83.6–103.6	96.4–105.6
Tyramine	94.2–102.8	92.2–95.5	82.6–92.0	72.0–91.0
Spermidine	70.0–77.7	70.0–86.3	76.0–95.4	78.0–87.0
Spermine	69.0–72.3	73.5–87.8	79.3–93.5	70.1–74.5

and 100 % despite some exceptions. The lowest recoveries were found for spermine and spermidine usually below 90 mg/kg with the exception of smoked salmon using 100 mg/kg spiking level. Low recoveries for all amines were also observed using marinated samples that might be affected by the low pH of the products.

Marinated fish products

Table 3 shows biogenic amine contents of marinated fish products. Only three samples contained histamine values over 50 mg/kg, which is the permitted level set by FDA [6]. According to EU regulations, two samples from Turkish producers were found above the permitted levels. On the other hand, these samples were found to be within the acceptable limits set by Turkish authorities. The study of Pechanek et al. [28] with commercial marinated samples also supported our results for histamine levels. The contents of other biogenic amines were found to be below 86.4 mg/kg, with the highest level represented by cadaverine.

Several factors have been reported to contribute to histamine development in marinated fish products [11, 29]. They may be summarized as follows: (1) amino acid decarboxylase activity is higher in an acidic environment and bacteria are more strongly encouraged to produce decarboxylase, (2) tissue cathepsins are more active in acidic conditions and result in the degradation of some muscle proteins into peptides and amino acids which are the precursors of amine formation in the presence of amino acid decarboxylase-positive microorganisms [11, 29]. Since pH affects the susceptibility to microbial growth, acidification is used in the preservation of many foods, including fish products [30]. Table 4 represents the pH levels of marinated samples. The values were found lower than 4.5 with most samples. Varying levels of pH were also observed in different studies for marinated fish products depending on storage conditions, packaging methods and

the concentration of acid and salt used, the presence of other ingredients and processing techniques [11, 30].

Since past studies indicated that histamine formation is expected to be high in marinated fish products because decarboxylase activity is higher in acidic environment and other favourable conditions [11, 29], chill storage below 2 °C is advised and commonly used in seafood-processing companies [1]. Gökoğlu [11] demonstrated that histamine and some other biogenic amines in two different marinated sardine products reached unsafe levels within a day during maturation at ambient temperature (25 ± 2 °C) with a variation according to the acetic acid levels used for marinating process. They also observed a decrease in the contents of tyramine, putrescine and histamine in the first 2–3 months of storage, followed by continuous increase up to 5 months of the storage.

Salt contents seen in the present study varied between 3.0 and 6.3 %, while %WPS accounted for 4.9–10.3 % depending on various moisture contents (Table 4). The a_w levels were also affected by the presence of salt content. Therefore, high a_w levels were observed, usually over 0.9. The a_w levels in marinated fish products were not found low enough to support microbial damage or avoid growth of pathogenic microorganisms as also pointed out by Cabrer et al. [31]. On the other hand, the combined effect of a_w and low pH places these products in the inhibition zone to growth of pathogenic bacteria and many of the deteriorant microorganisms [31]. Szymczak et al. [32] reported that most palatable marinades contained 2–3 % salt in the fish meat. On the other hand, marinades with higher salt content over 10 % were also commonly processed.

It was pointed out that microbial degradation of the proteins is not possible because of the high sensibility of microorganisms to acid medium [31]. However, various bacteria are reported to grow in marinated fish products, some being demonstrated as spoilage microorganisms such as homo and heterofermentative lactobacilli [31, 33].

Table 3 Biogenic amine contents of various marinated fish products originated from some European countries and Turkey

No	Sample type, company no and country origin	Levels of biogenic amines (ppm)									
		HIS	TRP	PHE	PUT	CAD	TYR	SPD	SPM		
1	Anchovy in oil (in PB)-C1A, Turkey	1.2 ± 0.1	15.7 ± 0.8	7.3 ± 0.6	3.6 ± 0.6	24.9 ± 1.1	10.0 ± 0.2	12.2 ± 0.9	2.5 ± 0.1		
2	Anchovy in oil (in PB)-C1B, Turkey	3.2 ± 0.1	NA	NA	4.0 ± 0.1	6.9 ± 0.1	NA	NA	NA		
3	Anchovy in oil (in PB)-C1C, Turkey	2.9 ± 0.1	22.9 ± 0.3	2.1 ± 0.1	3.4 ± 0.1	10.1 ± 0.5	4.9 ± 0.0	12.5 ± 0.6	2.0 ± 0.1		
4	Anchovy (sold in bulk at supermarket and repacked in PB)-C1D, Turkey	106.1 ± 3.7	5.5 ± 0.4	4.9 ± 0.1	1.8 ± 0.1	2.8 ± 0.2	3.9 ± 0.2	79.1 ± 0.5	3.6 ± 0.2		
5	Anchovy (sold in bulk at supermarket & repacked in PB)-C2, Turkey,	2.6 ± 0.4	4.2 ± 5.6	7.5 ± 0.6	2.9 ± 0.1	1.3 ± 0.2	3.5 ± 0.5	23.8 ± 0.4	1.9 ± 0.2		
6	Anchovy (in Mediterranean sauce & in plastic VP)-C3, Turkey	3.5 ± 0.2	10.3 ± 1.6	6.1 ± 0.3	2.3 ± 0.0	7.7 ± 0.3	3.0 ± 0.1	9.8 ± 1.8	0.9 ± 0.2		
7	Anchovy (in PB)-C4, Italy	2.0 ± 0.1	55.8 ± 2.9	3.6 ± 0.4	10.5 ± 1.5	7.6 ± 1.0	3.0 ± 0.7	15.4 ± 3.9	2.0 ± 0.3		
8	Anchovy (in Mediterranean style herbal sauce & PB)-C1E, Turkey	2.1 ± 0.1	11.6 ± 0.4	4.3 ± 0.1	3.7 ± 0.0	10.1 ± 0.2	5.1 ± 0.3	10.8 ± 0.3	ND		
9	Anchovy (in oil with red pepper, packed in PB)-C1F, Turkey	32.2 ± 0.8	16.5 ± 0.5	6.5 ± 0.6	14.7 ± 0.6	35.9 ± 1.1	22.0 ± 1.3	12.3 ± 0.6	2.2 ± 0.1		
10	Anchovy (in oil & herbal sauce, in plastic VP)-C3G, Turkey	93.8 ± 0.5	7.5 ± 0.6	3.7 ± 0.2	0.5 ± 0.6	1.6 ± 0.2	2.5 ± 0.6	6.0 ± 0.3	1.4 ± 0.3		
11	Anchovy (wrapped around green olives, in PB)-C1H, Turkey	2.0 ± 0.2	12.0 ± 1.0	8.1 ± 0.2	1.8 ± 0.1	2.9 ± 0.1	3.1 ± 0.3	11.2 ± 0.7	1.8 ± 0.1		
12	Anchovy (with garlic in PB)-C1I, Turkey	157.9 ± 1.6	7.5 ± 0.4	8.0 ± 0.3	15.5 ± 0.3	86.4 ± 0.4	59.2 ± 1.3	18.8 ± 0.3	2.9 ± 0.1		
13	Herring (with taste of smoked salmon & spices, in VP)-C5, Romania	1.3 ± 0.0	27.0 ± 0.2	3.1 ± 0.3	0.2 ± 0.1	5.2 ± 1.6	46.8 ± 0.6	9.2 ± 0.2	0.6 ± 0.1		
14	Mackerel (in oil & PB)-C1 J, Turkey	1.9 ± 0.1	6.3 ± 0.2	19.2 ± 0.3	1.6 ± 0.1	1.4 ± 0.1	3.7 ± 0.1	26.4 ± 0.6	2.4 ± 0.1		
15	Çiroz (smoked & marinated mackerel in oil, with pickles in PB)-C1 K, Turkey	1.4 ± 0.4	4.8 ± 0.1	3.9 ± 0.2	13.2 ± 0.2	5.0 ± 0.2	6.2 ± 0.1	19.4 ± 0.3	2.0 ± 0.1		
16	Çiroz (smoked & marinated mackerel in oil & PB)-C1L, Turkey	1.9 ± 0.1	17.8 ± 0.4	12.7 ± 0.3	15.0 ± 0.4	2.4 ± 0.3	8.4 ± 0.7	19.4 ± 0.2	2.2 ± 0.0		
17	Salmon (in oil, with pickles packed in PB)-C6, Italy	9.1 ± 0.1	6.1 ± 0.1	2.8 ± 0.0	2.8 ± 0.1	11.5 ± 0.1	17.7 ± 0.1	19.9 ± 0.2	2.1 ± 0.0		
18	Sardine (in oil & GJ)-C7, Italy	ND	5.1 ± 0.1	ND	ND	0.6 ± 0.0	1.7 ± 0.0	0.7 ± 0.0	0.9 ± 0.0		
19	Tuna (in VP)-C8, Slovenia	ND	4.7 ± 0.1	ND	ND	0.4 ± 0.0	1.6 ± 0.0	1.0 ± 0.0	1.5 ± 0.0		
20	Tuna (in VP)-C9, Greece	7.9 ± 0.2	6.6 ± 0.1	ND	0.9 ± 0.0	4.0 ± 1.2	6.5 ± 0.6	22.2 ± 0.6	8.1 ± 0.1		

±, SD, NA not analysed due to limitation in sample amount or other conditions. All the samples originated from fish-processing companies and they were stored at refrigerator

PB plastic box, VP vacuum pack, GJ glass jar, C1, C2, C3... shows the company numbers. A, B, C, D, E represents the different products or samples from different lots of the same company

HIS Histamine, TRP Triptamine, PHE Phenethylamine, PUT Putrescine, CAD Cadaverine, TYR Tyramine, SPD Spermidine, SPM Spermine

ND not detected (The levels were under detection limit: HIS = 0.85; TRP = 1.80; PHE = 1.51; PUT = 0.57; CAD = 0.67; SPM = 0.72). NA not analysed, HIS Histamine, TRP Triptamine, PHE Phenethylamine, PUT Putrescine, CAD Cadaverine, TYR Tyramine, SPD Spermidine, SPM Spermine

Table 4 Physical, chemical and microbiological properties of marinated fish products originated from some European countries and Turkey in relation to seafood safety

No ^a	Sample type	pH	a_w	Moisture (%)	Salt (%)	WPS (%)	TVC	HFB	THB
1	Anchovy, Turkey	4.55 ± 0.06	0.972 ± 0.001	59.0 ± 0.2	4.7 ± 0.0	7.4 ± 0.1	2.17 ± 0.03	NO	1.95 ± 0.03
2	Anchovy, Turkey	3.88 ± 0.07	0.957 ± 0.000	57.7 ± 1.1	6.0 ± 0.0	9.4 ± 0.1	<1.47	<1.47	NA
3	Anchovy, Turkey	4.31 ± 0.07	0.947 ± 0.001	56.8 ± 0.2	4.9 ± 0.1	7.9 ± 0.2	NA	NA	NA
4	Anchovy, Turkey	4.17 ± 0.09	0.972 ± 0.001	59.1 ± 1.1	3.0 ± 0.2	4.9 ± 0.3	3.28 ± 0.03	3.04 ± 0.06	NA
5	Anchovy, Turkey	3.88 ± 0.03	0.927 ± 0.001	56.7 ± 1.1	4.2 ± 0.3	7.0 ± 0.6	NA	NA	NA
7	Anchovy, Italy	4.23 ± 0.10	0.947 ± 0.001	62.3 ± 0.3	4.1 ± 0.1	6.2 ± 0.2	NA	NA	NA
8	Anchovy, Turkey	3.93 ± 0.04	0.966 ± 0.001	54.5 ± 0.4	5.7 ± 0.2	9.4 ± 0.3	2.90 ± 0.02	2.54 ± 0.05	2.14 ± 0.03
9	Anchovy, Turkey	4.11 ± 0.02	0.958 ± 0.001	64.3 ± 0.4	6.3 ± 0.2	9.0 ± 0.2	1.77 ± 0.01	NO	1.60 ± 0.01
10	Anchovy, Turkey	3.51 ± 0.06	0.914 ± 0.001	54.4 ± 0.7	5.7 ± 0.1	9.5 ± 0.1	3.75 ± 0.09	3.51 ± 0.07	NA
11	Anchovy, Turkey	3.61 ± 0.10	0.964 ± 0.001	66.0 ± 0.5	4.9 ± 0.1	6.9 ± 0.2	<1.47	<1.47	NA
12	Anchovy, Turkey	3.90 ± 0.05	0.968 ± 0.001	65.0 ± 1.3	5.2 ± 0.2	7.4 ± 0.4	3.51 ± 0.06	3.38 ± 0.09	NA
13	Herring, Romania	4.38 ± 0.02	0.931 ± 0.001	64.2 ± 1.4	5.5 ± 0.2	7.9 ± 0.1	NA	NA	NA
14	Mackerel, Turkey	5.30 ± 0.02	0.945 ± 0.001	48.5 ± 0.6	4.5 ± 0.1	8.5 ± 0.2	NA	NA	NA
15	Mackerel (çiroz), Turkey	5.17 ± 0.01	0.952 ± 0.001	51.5 ± 0.5	4.0 ± 0.0	7.2 ± 0.0	2.38 ± 0.02	2.32 ± 0.05	2.43 ± 0.02
16	Mackerel (çiroz), Turkey	5.30 ± 0.02	0.945 ± 0.001	51.5 ± 0.6	4.5 ± 0.0	8.5 ± 0.2	2.34 ± 0.03	NO	2.25 ± 0.02
17	Salmon, Italy	5.12 ± 0.01	0.897 ± 0.001	60.9 ± 1.6	5.1 ± 0.0	7.8 ± 0.2	NA	NA	NA
18	Sardine, Italy	4.10 ± 0.10	0.957 ± 0.001	61.3 ± 1.3	4.8 ± 0.1	7.3 ± 0.3	NA	NA	NA
19	Tuna, Slovenia	5.89 ± 0.01	0.922 ± 0.001	42.4 ± 1.4	4.8 ± 0.1	10.3 ± 0.4	3.04 ± 0.06	2.81 ± 0.04	NO
20	Tuna, Greece	4.58 ± 0.02	0.923 ± 0.001	62.2 ± 1.4	6.3 ± 0.2	9.2 ± 0.4	NA	NA	NA

All the samples originated from fish-processing companies and they were stored at a refrigerator at 4 °C ± 1

±, SD, NA not analysed, NO no observation, TVC total viable counts, HFB Histamine-forming bacteria, THB total halophilic bacteria, WPS water phase salt

^a Sample no represents the same numbers stated at Table 1 (Missing numbers indicates that the sample was not analysed due to sample limitation)

Therefore, marinated fish products have a limited shelf-life [33], and cold storage is necessary to avoid spoilage and development of biogenic amines. Fuselli et al. [33] showed that bacteria not completely inactivated after the marinating are able to grow during storage according to their ability to adapt in the acid medium. They reported that the a_w and pH values in marinated fish products allowed the growth of various types of bacteria. Our study also supported these results in terms of bacteria survival/growth, and varying numbers of bacteria were observed in marinated fish products. Total viable bacteria, total histamine-forming bacteria and total halophilic bacteria counts ranged from <1.47 to 3.75, no-detection (NO) to 3.51 and NO to 2.43 log cfu/g, respectively. The counts of total viable and total histamine-forming bacteria over 3 log cfu/g represented by the samples containing high histamine values (93 mg/kg and over), suggesting strong bacteria support at favourable conditions during processing and storage of marinated fish products for histamine formation as also suggested by past studies mentioned above. Previous studies demonstrated that acidification selects for acid-

tolerant bacterial species in marinated fish, and onboard handling of raw materials did no influence flora development in these products [33].

Smoked fish products

Table 5 represents biogenic amine contents of smoked fish products. Only one sample contained high levels of histamine at 99 mg/kg, which is over FDA permitted level and close to EU but lower than Turkish permitted values. Low histamine values in the products indicate good manufacturing and/or good hygienic practices (GMP/GHP) and Hazard Analysis Critical Control Points (HACCP) applications in these factories are usually properly applied. There have been several reports on high histamine levels in smoked fish products which exceed either the FDA or EU permitted amounts [3, 14, 28, 33]. Lehane and Olley [3] indicated that hot-smoking ‘practically sterilizes’ the product and denatures enzymes, imparting some degree of preservation but does not destroy histamine already formed. However, such products are susceptible to histamine

Table 5 Biogenic amine contents of various smoked products in vacuum packs originated from some European countries and Turkey

No	Sample type, company no and country origin	Levels of biogenic amines (ppm)							
		HIS	TRP	PHE	PUT	CAD	TYR	SPD	SPM
1	Cold-smoked Salmon, C1, France	ND	4.7 ± 0.1	0.1 ± 0.0	ND	79.8 ± 0.2	3.7 ± 0.0	0.3 ± 0.0	1.1 ± 0.0
2	Cold-smoked Salmon, C2, Norway	ND	4.6 ± 0.1	0.4 ± 0.0	ND	22.6 ± 0.9	2.9 ± 0.1	0.2 ± 0.0	1.0 ± 0.0
3	Cold-smoked Salmon, C3, Norway	2.7 ± 0.1	8.8 ± 0.3	15.1 ± 0.5	6.7 ± 0.2	38.8 ± 1.0	460.9 ± 10.2	7.8 ± 0.3	5.3 ± 0.3
4	Cold-smoked Salmon, sliced, C4, Norway	1.8 ± 0.1	19.0 ± 0.2	29.1 ± 0.5	146.0 ± 3.5	13.0 ± 0.8	272.5 ± 6.0	8.8 ± 0.2	4.7 ± 0.2
5	Cold-smoked Salmon, C5, Scotland	ND	4.4 ± 0.1	0.5 ± 0.0	ND	5.5 ± 0.6	1.7 ± 0.1	0.3 ± 0.0	1.0 ± 0.0
6	Cold-smoked Salmon, C6, Scotland	30.9 ± 0.4	10.0 ± 0.3	61.0 ± 1.6	318.0 ± 10.4	16.6 ± 0.2	416.7 ± 16.1	5.6 ± 0.4	3.7 ± 0.1
7	Cold-smoked Salmon, C7, Turkey	1.5 ± 0.1	NA	NA	2.5 ± 0.1	ND	NA	NA	NA
8	Hot-smoked Anchovy, C8A, Turkey	1.8 ± 1.1	NA	NA	8.3 ± 0.3	0.5 ± 0.6	NA	NA	NA
9	Hot-smoked Anchovy, C8B, Turkey	1.9 ± 0.1	5.3 ± 0.2	10.3 ± 0.6	2.7 ± 0.2	ND	0.7 ± 0.1	8.3 ± 0.2	0.6 ± 0.7
10	Hot-smoked Anchovy, C8C, Turkey	7.7 ± 0.3	33.0 ± 1.3	7.6 ± 0.4	10.4 ± 0.7	1.2 ± 0.2	12.5 ± 0.8	15.2 ± 1.1	2.1 ± 0.3
11	Hot-smoked & M Anchovy, C8D, Turkey	1.9 ± 0.0	5.6 ± 0.0	18.4 ± 0.0	1.0 ± 0.0	14.6 ± 0.5	2.4 ± 0.0	15.2 ± 0.3	1.7 ± 0.1
12	Hot-smoked Bonito, C9A, Turkey	11.2 ± 0.1	NA	NA	2.1 ± 0.1	7.4 ± 0.4	NA	NA	NA
13	Hot-smoked Bonito, C9B, Turkey	98.7 ± 0.6	187.0 ± 1.2	132.4 ± 0.7	211.7 ± 0.8	1,862.0 ± 5.9	783.7 ± 0.8	5.6 ± 0.6	6.2 ± 0.2
14	Hot-smoked Bonito, C10, Turkey	2.7 ± 0.3	6.5 ± 0.5	20.1 ± 0.6	1.6 ± 0.1	1.7 ± 0.0	14.8 ± 0.5	79.1 ± 0.9	7.6 ± 0.1
15	Hot-smoked Bonito, C11, Turkey	3.0 ± 0.1	5.5 ± 0.2	5.3 ± 0.3	1.8 ± 0.1	41.2 ± 0.9	3.5 ± 0.2	76.8 ± 1.3	3.5 ± 0.3
16	Cold-smoked Tuna fillets, C12, Lithuania	ND	7.0 ± 0.2	123.2 ± 2.1	0.9 ± 0.0	30.9 ± 1.7	21.7 ± 1.3	50.3 ± 2.8	5.3 ± 0.2
17	Cold-smoked Tuna flesh, C13, Italy	ND	ND	4.7 ± 0.3	ND	4.0 ± 0.1	1.5 ± 0.1	2.0 ± 0.0	31.2 ± 0.6
18	Hot-smoked Mackerel, C9C, Turkey	1.1 ± 0.2	NA	NA	3.1 ± 0.1	ND	NA	NA	NA
19	Hot-smoked Herring, C9D, Turkey	1.3 ± 0.1	10.6 ± 0.1	2.9 ± 0.1	3.5 ± 0.3	ND	6.9 ± 0.3	9.4 ± 0.5	1.9 ± 0.1

Table 5 continued

No	Sample type, company no and country origin	Levels of biogenic amines (ppm)							
		HIS	TRP	PHE	PUT	CAD	TYR	SPD	SPM
20	Hot-smoked Trout, C9E, Turkey	3.3 ± 0.2	7.2 ± 0.2	16.1 ± 0.6	4.3 ± 0.0	8.3 ± 0.2	35.8 ± 0.6	26.9 ± 0.5	7.3 ± 0.1

C1, C2, C3... shows the company numbers. A, B, C, D, E represents the different products or samples from different lots of the same company. S&M smoked & marinated

±, SD All the samples originated from fish-processing companies and they were stored at refrigerator. NA Not analysed due to limitation in sample amount or other conditions

HIS Histamine, TRP Triptamine, PHE Phenylethylamine, PUT Putrescine, CAD Cadaverine, TYR Tyramine, SPD Spermidine, SPM Spermine
ND not detected (The levels were under detection limit: HIS = 0.85; TRP = 1.80; PHE = 1.51; PUT = 0.57; CAD = 0.67; SPM = 0.72)

production if recontamination occurs due to poor handling during packaging and they are stored at elevated temperatures [1]. A recent review from New Zealand confirms this statement since 24 outbreaks relating to histamine poisoning were reported caused by hot smoked fish between 2000 and 2009 [4]. It also indicated that New Zealand outbreaks and product recalls were dominated by hot smoked fish. The samples in the present study were both hot and cold-smoked types, and all of them were stored in vacuum pack at cold-storage temperature. Emborg et al. [34] linked high concentration of histamine in vacuum-packed tuna with the growth of psychrotolerant *Morganella morganii*-like bacteria or with *Photobacterium phosphoreum*. A number of the histamine-forming bacteria are known to be facultative anaerobes that can grow in reduced oxygen environments [35]. Since such bacteria can also grow at refrigerated temperatures, vacuum packing of both cold and hot-smoked fish is at risk for histamine-forming if GMP/GHP are not applied [1].

Jørgensen et al. [12] reported that the production of biogenic amines in cold-smoked salmon during chill storage is unlikely to result in histamine poisoning in humans due to previous epidemiological studies. Moreover, it was pointed out that salmon was the fish species least susceptible to histamine formation, probably due to low content of free histidine in the muscle [36]. On the other hand, the study of Emborg et al. [34] shows that this situation may depend on the type of histamine-forming bacteria present in the product before and after processing. Other bacteria-growth promoting factors can also affect the histamine formation.

In the present study, five samples contained high levels of other biogenic amines in smoked fish products from ND up to 1,862.0 mg/kg (Table 5), the highest level represented by tyramine. Although toxicity levels and acceptable limits set for histamine by the regulatory authorities, no limits were known for other biogenic amines despite their potentiating or synergistic role in histamine food

poisoning or direct toxicity affect of some amines reported in the literature [3]. Cadaverine and putrescine are known to potentiate histamine toxicity [3] and taking a role in nitrosamine formation in fish products [37]. Therefore, high putrescine levels are also important for food safety. Lawley et al. [38] reported that tyramine has similar acute toxicity with cadaverine and putrescine and has been associated with hypertension and headaches in sensitive individuals, especially those suffering serious migraines [39]. Moreover, phenylethylamine has also been reported to cause headache, migraine and hypertension. Limited studies exist on biogenic amines other than histamine contents in smoked fish products. Montiel et al. [40] and Dondero et al. [36] obtained low levels of biogenic amines in smoked fish products (cod and salmon species). However, the values increased at the end of the different cold-storage periods [36]. Jørgensen et al. [12] observed a correlation between the concentrations of biogenic amines and the development of spoilage off-flavours for cold-smoked vacuum-packed salmon.

The salt contents in the present study for smoked fish products varied as 2.5–14.3 % (Table 6). The principal food safety parameter in cold-smoked fish was reported as the amount of %WPS in the finished product [12]. Varying %WPS levels were observed in the current study in the range of 3.6–23.0 %. European products usually contained lower salt contents in comparison with Turkish ones. FDA [41] suggest a minimum WPS level of 3.5 or 3.0 % with not less than 100 mg/kg nitrite for refrigerated, reduced oxygen packaged (e.g. vacuum or modified atmosphere packaged) smoked fish or smoke-flavoured fish. Jørgensen et al. [12] found a close correlation between a_w and WPS for cold-smoked salmon and suggested that the measurement of a_w can replace the laborious analysis of salt content and dry matter used for verification of the salting process of cold-smoked salmon.

Jørgenson et al. [12] reported %WPS and a_w levels of cold-smoked salmon ranging as 3.0–12.6 % WPS and

0.92–0.98 a_w , respectively. The data from vacuum-packed cold-smoked salmon in the present study compared well with the results of Jørgenson et al. [12]. Goulas and Kontominas [42] reported that moisture contents can vary according to smoking methods and, observed 56.7 and 56.9 % moisture for vacuum-packed chub mackerel. Moisture contents in our study were in a wide range of 48.1–71.2 %. The pH of the smoked fish products is known to support bacterial growth and the values were found between 5.29 and 6.53. Jørgenson et al. [12] obtained initial pH for cold-smoked salmon at 6.00–6.16, and the levels changed during storage period at 5.93–6.10.

The results of bacteria counts are given in Table 6. Although the counts of total viable and histamine-forming bacteria were found in the wide range, low levels of halophilic bacteria counts were observed <1.47 log cfu/g, indicating either the salt level in the products or time after salting was not enough to support their growth. Other factors such as heating and smoke components can also be effective to destroy bacteria or inhibit their growth. Al-Bulushi et al. [37] reported that mesophilic bacterial count of log 6–7 cfu/g has been associated with 50 mg/kg histamine. Our results were lower than the suggested value for the histamine health risk. The counts of histamine-forming bacteria do not also support the histamine levels in the analysed products as observed for marinated

samples. Dondero et al. [36] observed higher total viable counts in cold-smoked salmon. González-Rodríguez et al. [43] pointed out that the smoking process provides some antimicrobial action but during subsequent refrigerated storage, the surviving microflora multiply and cause spoilage. If packaging limits available oxygen, microorganisms capable of anaerobic respiration are predominant. Under these conditions, lactic acid bacteria typically dominate the microflora. Various studies demonstrated the growth and survival of histamine formers and spoilage bacteria in smoked fish products [34, 36]. Dondero et al. [36] pointed out that during cold-smoking of salmon, the temperature never exceeds 28 °C; therefore, there is no heat inactivation of native enzymes in the tissue, this situation will also allow previously formed enzymes for amine forming by the amine-forming bacteria. FDA [41] pointed out that hot-smoking helps to reduce the pathogenic bacteria but also spoilage bacteria. The spoilage organisms would otherwise have competed with and inhibited the growth of *C. botulinum*. On the other hand, in cold-smoked fish, it is important that the product does not receive so much heat that the number of spoilage organisms is significantly reduced. Therefore, these spoilage organisms must be present to inhibit the growth and toxin formation of *C. botulinum* type E and non-proteolytic types B and F.

Table 6 Physical, chemical and microbiological properties of smoked fish products originated from some European countries and Turkey in relation to seafood safety

No ^a	Sample type	pH	a_w	Moisture (%)	Salt (%)	WPS (%)	TVC	HFB	THB
1	Salmon, France	6.02 ± 0.02	0.946 ± 0.001	67.8 ± 0.3	5.3 ± 0.1	7.2 ± 0.1	3.40 ± 0.05	3.20 ± 0.06	NO
2	Salmon, Norway	6.16 ± 0.02	0.967 ± 0.002	71.2 ± 0.0	3.6 ± 0.1	4.8 ± 0.1	4.04 ± 0.05	3.46 ± 0.06	NO
3	Salmon, Norway	6.53 ± 0.06	0.957 ± 0.001	65.7 ± 0.6	2.5 ± 0.2	3.6 ± 0.2	4.00 ± 0.07	3.93 ± 0.06	NO
4	Salmon, sliced, Norway	6.13 ± 0.05	0.952 ± 0.001	66.3 ± 0.2	4.2 ± 0.2	4.0 ± 0.1	NA	NA	NA
5	Salmon, Scotland	6.17 ± 0.01	0.961 ± 0.001	66.0 ± 0.1	4.1 ± 0.0	5.9 ± 0.0	3.48 ± 0.08	3.45 ± 0.02	NO
6	Salmon, Scotland	6.20 ± 0.07	0.961 ± 0.001	67.2 ± 0.9	4.2 ± 0.2	5.8 ± 0.1	2.04 ± 0.05	1.95 ± 0.05	NO
7	Salmon, Turkey	6.30 ± 0.01	0.961 ± 0.001	55.2 ± 1.2	5.1 ± 0.1	8.5 ± 0.3	NA	NA	NA
9	Anchovy, Turkey	5.57 ± 0.04	0.933 ± 0.001	51.3 ± 1.2	5.9 ± 0.1	10.3 ± 0.3	NA	NA	NA
10	Anchovy, Turkey	5.29 ± 0.08	0.936 ± 0.001	52.2 ± 1.0	4.0 ± 0.1	7.1 ± 0.1	<1.47	<1.47	NO
12	Bonito, Turkey	6.03 ± 0.02	0.953 ± 0.002	52.3 ± 0.6	6.2 ± 0.1	10.7 ± 0.2	NA	NA	NA
15	Bonito, Turkey	5.88 ± 0.11	0.950 ± 0.001	57.9 ± 1.4	5.0 ± 0.1	7.9 ± 0.0	3.98 ± 0.06	3.85 ± 0.11	NO
16	Tuna, Turkey	5.89 ± 0.11	0.952 ± 0.001	54.0 ± 0.5	6.8 ± 0.4	11.2 ± 0.8	<1.47	<1.47	NO
17	Tuna, Turkey	6.21 ± 0.06	0.959 ± 0.000	52.1 ± 0.2	4.2 ± 0.1	7.5 ± 0.2	2.70 ± 0.04	2.36 ± 0.06	NO
18	Mackerel, Turkey	5.92 ± 0.08	0.926 ± 0.001	48.4 ± 0.3	5.3 ± 0.1	9.9 ± 0.2	2.49 ± 0.02	2.41 ± 0.03	<1.47
19	Herring, Turkey	6.07 ± 0.02	0.786 ± 0.001	48.1 ± 0.2	14.3 ± 0.3	23.0 ± 0.4	2.17 ± 0.03	2.39 ± 0.04	NO
20	Trout, Turkey	6.09 ± 0.06	0.947 ± 0.001	64.0 ± 0.6	6.7 ± 0.5	9.4 ± 0.5	3.53 ± 0.02	3.49 ± 0.03	NO

All the samples originated from fish-processing companies and they were stored at refrigerator 4 °C ± 1

±, SD, NA not analysed, NO no observation, TVC total viable counts, HFB histamine-forming bacteria, THB total halophilic bacteria, WPS water phase salt

^a Sample no represents the same numbers stated at Table 1 (Missing numbers indicates that the sample was not analysed due to sample limitation)

Fermented fish products

Table 7 shows biogenic amine levels for various fermented fish products. In Europe, fermentation of fish has a long history, however, nowadays mainly associated with the Scandinavian countries [44]. Knochel [45] described traditional fermented fish products in Scandinavia and she reported that two types of fermented fish products, namely ‘gravad fisk’ also known as ‘gravadlax’ and ‘Kryddersild’ are widely accepted in these countries. Another type of fermented fish product is called ‘Surströmming’ which is a Swedish product made of Baltic herring [45]. EU and Turkish authorities allow histamine levels at 200 mg/kg (2 samples/9 at 400 mg/kg) in fermented fish products [8, 9], while FDA allows less than 500 mg/L for fish sauce [7]. *Surströmming* samples contained histamine within the acceptable values. The levels of other biogenic amines of these products were usually found lower than 50 mg/kg except for cadaverine. This situation suggests that cadaverine in *surströmming* products may potentiate histamine health risk or be involved in nitrosamine formation as pointed out by various researchers [3, 37]. Low levels of histamine and other biogenic amines were observed for *Kryddersild* products analysed in this study. The higher biogenic amine contents in *surströmming* samples in comparison with ‘kryddersild’ may have been caused due to storage temperature during maturation. Low salt levels may have also contributed in high BAs for some samples.

Lyhs et al. [46] reported that sugar-salted fish products are accepted as ‘gravadlax’ and are known as very popular dishes traditionally manufactured in the Nordic countries. This product is matured and stored at 5 °C [45]. The *gravadlax* sample from Scotland contained low levels of histamine and other biogenic amines. However, high level of histamine was found for the sample originated from Turkey. The other biogenic amines were also observed high in a range of 81.2–969.4 mg/kg with the exceptions of spermidine and spermine. The high levels might have been caused due to either poor raw material quality and/or improper processing because of unfamiliarity with this technique in this country. No study exists on the biogenic amines contents of this type of product; therefore, comparison was not possible.

High histamine values were observed from four fish paste products obtained from Turkey, Greek and Italy; however, only one sample from Greece exceeded the EU-permitted level. Two Turkish samples were also found close to accepted limits. The levels of other BAs were found less than 100 mg/kg with the exception of tryptamine.

Østergaard et al. [47] pointed out that the fermentation process for fish may fulfil the conditions required for abundant formation of biogenic amines, such as availability of free amino acids, presence of decarboxylase-positive

microorganisms and conditions allowing bacterial growth, which have decarboxylase synthesis and decarboxylase activity. Studies related to histamine in fermented fish products usually cover the products of Southeast Asian or African origin and high histamine levels were reported particularly for fish sauce products [5, 7, 17]. Mah et al. [15] indicated that different types of fermented fish products may contain different biogenic amine levels depending on the species and methodology used. They also further demonstrated rise in the levels considerably during storage for longer than 10 days at 4, 10 and 15 °C. Brillantes et al. [13] found a good correlation between histamine levels in raw material and final products, and speculated that formation of histamine occurred by histidine decarboxylase which might have formed prior to fermentation. Various past studies observed high biogenic amine levels in different commercial fish paste products originated from different countries [18].

The main difficulty in preventing histamine formation in Southeast Asian products comes from the fermenting process usually applying ambient temperature conditions and processing techniques involving bacterial enzymes originated from fish [1]. Due to the difficulty in preventing histamine formation in most Southeast Asian fermented fish products, especially in fish sauce, several studies are concentrated on decreasing histamine levels using the inhibition effect of additives or using other degradative compounds [35, 48, 49]. For European fermented fish products, using chill storage and proper handling of raw materials can be effective methods of preventing biogenic amine development, although the risk relating high content of cadaverine must be further investigated. Various authors reported the effect of mixed starter bacteria cultures with negative-decarboxylate activity on decreasing histamine and some other biogenic amines such as tyramine [16, 35, 50]. Sanceda et al. [51] demonstrated that although histamine levels in fish sauce increase during fermentation, a decrease occurs after certain levels depending on the salt concentration that might also explains the low levels in the commercial samples. Kuda et al. [52] investigated histamine content and numbers of histamine-forming and histamine-decomposing bacteria in 10 fish-*nukazuke* (a Japanese uncooked, salted and fermented fish with rice-bran) products. Their results revealed that histamine content tended to be low in the product containing high number of halophilic histamine-decomposing bacteria.

The levels of pH, a_w , %salt, %WPS and %moisture for fermented fish products in the present study varied in great range (Table 8). Generally, pH should be below 5.0–4.5 to inhibit pathogenic and spoilage bacteria [53]. Therefore, pH values of all fermented fish products in our study are likely to promote growth of either pathogenic and/or spoilage bacteria. Mah and Hwang [48] reported that the

Table 7 Biogenic amine contents of various fermented fish products of some European countries and Turkey

No	Sample type, company no and country origin	Levels of biogenic amines (ppm)							
		HIS	TRP	PHE	PUT	CAD	TYR	SPD	SPM
1	Gravadlax (M. F. Salmon, in VP), C1, Scotland	23.1 ± 1.2	5.0 ± 0.1	6.6 ± 0.7	9.2 ± 0.9	32.5 ± 4.6	73.3 ± 0.4	6.0 ± 0.4	7.1 ± 0.2
2	Gravadlax (S.F. Salmon, in VP), C2, Turkey,	529.3 ± 14.1	81.2 ± 2.1	219.0 ± 7.1	192.0 ± 4.2	969.4 ± 12.7	456.2 ± 21.2	2.3 ± 0.2	2.7 ± 0.2
3	Fish Paste (Sardine, in MT), Italy	2.6 ± 0.1	11.3 ± 0.1	3.2 ± 0.0	2.2 ± 0.0	13.9 ± 0.0	7.9 ± 0.1	2.7 ± 0.1	2.2 ± 0.1
4	Fish paste (Tuna, in MT), C3A, Denmark	1.7 ± 0.0	5.5 ± 0.0	9.6 ± 0.1	2.9 ± 0.1	4.8 ± 0.1	3.1 ± 0.0	35.3 ± 0.2	4.9 ± 0.0
5	Fish paste (Tuna, in MT), C3B, Denmark	0.6 ± 0.1	6.0 ± 0.1	10.7 ± 0.2	2.6 ± 0.1	3.0 ± 0.2	3.2 ± 0.1	41.9 ± 2.8	4.0 ± 0.3
6	Fish paste (Salmon, in MT), C4, Germany	5.0 ± 0.1	7.1 ± 0.1	55.0 ± 4.6	1.1 ± 0.1	8.7 ± 0.1	44.7 ± 4.7	2.1 ± 0.1	1.4 ± 0.1
7	Fish paste (Anchovy, in MT), 5A, Turkey	191.7 ± 0.7	57.2 ± 1.9	34.9 ± 0.3	11.4 ± 0.5	67.5 ± 0.6	21.1 ± 0.2	9.4 ± 0.4	2.5 ± 0.0
8	Fish Paste (Anchovy, in MT), 5B, Turkey	2.4 ± 0.3	6.5 ± 0.4	4.5 ± 0.5	2.0 ± 0.1	1.3 ± 0.1	2.7 ± 0.4	39.2 ± 0.7	3.4 ± 0.2
9	Fish paste (Anchovy & Sardine, in MT), 5C, Turkey	165.9 ± 15.2	NA	NA	NA	NA	NA	NA	NA
10	Fish paste (Anchovy, in MT), C6, Greece	1,544.3 ± 1.2	517.2 ± 1.0	6.6 ± 0.5	11.9 ± 0.3	13.6 ± 0.4	18.6 ± 0.4	2.7 ± 0.1	2.0 ± 0.1
11	Fish paste (Anchovy, in MT), C7, Italy	3.8 ± 0.1	5.2 ± 0.1	5.7 ± 0.2	3.6 ± 0.0	21.7 ± 0.4	9.4 ± 0.2	7.5 ± 0.3	2.4 ± 0.3
12	Fish paste (Anchovy, in MT), C8, Italy	72.3 ± 1.2	NA	4.5 ± 0.2	5.5 ± 0.4	12.4 ± 0.5	6.4 ± 0.4	3.2 ± 0.4	NA
13	Surströmming ^a -C9A, Sweden	9.3 ± 0.4	28.7 ± 2.4	2.8 ± 0.5	20.9 ± 1.1	45. ± 1.5	29.6 ± 1.2	5.4 ± 0.4	2.0 ± 0.1
14	Surströmming-C9B, Sweden,	9.0 ± 0.4	10.7 ± 1.2	3.0 ± 0.8	15.9 ± 0.7	43.7 ± 0.5	30.5 ± 0.6	5.1 ± 0.9	2.1 ± 0.1
15	Surströmming (Fléer)-C10Ax, Sweden	46.1 ± 1.2	NA	NA	ND	571.6 ± 48.9	NA	NA	NA
16	Surströmming (Fléer)-C10Ay, Sweden	40.7 ± 9.4	NA	NA	NA	141.6 ± 0.6	NA	NA	NA
17	Surströmming (Erik den röde)-C10Bx, Sweden	38.6 ± 3.0	NA	NA	NA	NA	NA	NA	NA
18	Surströmming (Erik den röde)-C10By, Sweden	25.8 ± 7.4	NA	NA	NA	546.4 ± 10.0	NA	NA	NA

Table 7 continued

No	Sample type, company no and country origin	Levels of biogenic amines (ppm)							
		HIS	TRP	PHE	PUT	CAD	TYR	SPD	SPM
19	Surströmming (<i>Lekmogen</i>)- C10Cx, Sweden	25.4 ± 3.0	NA	NA	1.4 ± 0.1	NA	NA	NA	NA
20	Surströmming (<i>Lekmogen</i>)- C10Cy, Sweden	69.7 ± 2.4	NA	NA	10.4 ± 0.1	836.2 ± 28.7	NA	NA	NA
21	Surströmming (<i>Måsens</i>)-C 10Dx, Sweden	79.8 ± 3.1	NA	NA	ND	NA	NA	NA	NA
22	Surströmming (<i>Måsens</i>)-C 10Dy, Sweden	11.4 ± 0.4	ND	ND	35.7 ± 1.0	61.1 ± 1.1	33.5 ± 1.5	5.6 ± 0.9	NA
23	Kryddersild (F.B. herring, in PC)- C11A, Denmark	6.18 ± 0.3	2.2 ± 0.8	10.7 ± 1.2	15.3 ± 0.7	42.7 ± 0.5	28.8 ± 0.6	5.2 ± 0.9	1.4 ± 0.1
24	Kryddersild (F.B. herring in PC)- C1Bx, Denmark	8.6 ± 0.5	2.0 ± 1.2	1.9 ± 0.4	50.1 ± 1.9	24.8 ± 1.1	14.6 ± 0.5	13.0 ± 0.5	ND
25	Kryddersild (F. B. herring, in PC)- C11By, Denmark	9.0 ± 0.4	28.7 ± 2.4	2.0 ± 0.5	20.3 ± 1.1	44.1 ± 1.47	27.9 ± 1.2	5.3 ± 0.4	1.4 ± 0.1

All the samples originated from fish-processing companies and they were stored at refrigerator at 4 °C ± 1

VP vacuum pack, MT metal container, PC plastic container. C1, C2, C3... shows the company numbers. A, B, C, D, E represents the different products or samples from different lots of the same company. x, y represents the same products from the same lot of a company but analysed from different time within the shelf-life

±, SD. NA not analysed due to limitation in sample amount or other conditions

HIS Histamine, TRP Triptamine, PHE Phenethylamine, PUT Putrescine, CAD Cadaverine, TYR Tyramine, SPD Spermidine, SPM Spermine
ND not detected (The levels were under detection limit: HIS = 0.85; TRP = 1.80; PHE = 1.51; PUT = 0.57; CAD = 0.67; SPM = 0.72)

^a Surströmming: Fermented Baltic (F.B.) Herring in cans

optimum pH for histidine decarboxylase activity is acidic, ranging from 2.5 to 6.5. Although the pH levels of *surströmming* and *kryddersild* products were observed higher than the suggested optimum pH for histidine decarboxylase activity, most fish paste and *gravadlax* samples were within the mentioned optimum pH range that may explain the high histamine levels in some of these products. Lyhs et al. [46] reported that *gravadlax* fish products were characterized by a salt content of 3–6 % and a pH > 5. The characteristic pH values were also observed in the Scottish *gravadlax* samples in our study.

Kimura et al. [54] demonstrated the effect of pH on histamine formation in culture media using *Tetragenococcus muriaticus*, a halophilic lactic acid bacteria is isolated from fish sauce. Their results revealed that histamine formation was higher at pH 5.1–5.8 while lower histamine values were obtained at pH 7.1–7.6. Yongjin et al. [16] indicated that high temperature and low pH can accelerate amino acid accumulation and stimulate amine formation but, during fermentation processes, the factors affecting the

activity of the decarboxylating enzyme could be more important than the precursor availability.

Østergaard et al. [47] reported that fermented fish products are divided into two categories according to their salt content: the first is a high-salt (20–30 %) product in which the microbial count decreases during curing, and the second has a lower salt content to which fermentable carbohydrates are added. The latter category is characterized by an increase in microbial count during fermentation with a dominance of lactic acid bacteria. It was reported that levels of 3.5–5.5 % salt could inhibit histamine formation by *Klebsiella pneumoniae* and *Morganella morganii*, which are accepted as high histamine formers [13]. Salt concentration is also important in terms of promoting lactic acid bacteria formation in fermented fish products [53]. Kimura et al. [54] demonstrated that halophilic histamine-forming bacteria *T. muriaticus* proliferated in the medium at salt concentrations ranging from 3.0 to 20 % and histamine formation was optimal at a salt concentration of 5–7 %. Therefore, the inhibiting effect of salt on the

formation histamine and other biogenic amines may be more depending on the type of histamine-forming bacteria present in fermented fish products.

Salt content of the fermented fish and fish paste were above 3.5 %, on the other hand, the levels were found as 1.6 and 2.4 % for salt and WPS, respectively in the Turkish *gravadlax* sample (Table 8) explaining the high histamine and biogenic amine contents for this product. Salt concentration may also range widely depending on different types and batches of fermented fish products [53]. Knochel [45] stated that Scandinavian fermented fish products vary greatly in salt content such as ‘*gravad fish*’ having a rather low (9 % WPS) and ‘*kryddersild*’ a higher content (around 21 % WPS) during maturation. Except for Turkish *gravadlax* sample, our findings supported the statement of Knochel [45] for the relating fermented fish products. On the other hand, Lyhs et al. [46] reported that *gravadlax* products are characterized by a salt content of 3–6 %. Salt level of Turkish sample of the present study did not support

suggested levels indicating the change in the *gravadlax* procedures.

Since fermentation is carried out by lactic acid bacteria, most studies on this aspect were concentrated on the type of bacteria and their performance on fermentation. Some lactic acid bacteria, such as *Lactobacillus*, *Leuconostoc* and *Pediococcus*, which are used in fermenting fish, have been reported to form histamine or other lactic acid bacteria [50, 55]. Leisner et al. [55] identified *Carnobacterium* spp. as the major tyramine-producer for vacuum-packed sugar-salted salmon at 5 °C. Zarei et al. [17] suggested that high histamine content in a fish sauce product from Iran might be related to the high levels of bacterial count especially *Enterobacteriaceae* and lactic acid bacteria in this product. They obtained aerobic mesophilic counts in the range of 2.30–6.15, total halophilic bacteria at NO-4.68 log cfu/g. Brillantes et al. [13] observed a drop in total halophilic count during fermentation of anchovy fish sauce. Halophilic counts in the present study were also found low for

Table 8 Physical, chemical and microbiological properties of fermented fish products originated from some European countries and Turkey in relation to seafood safety

No ^a	Sample type	pH	a_w	Moisture (%)	Salt (%)	WPS (%)	TVC	HFB	THB
1	M. F. Salmon, Gravadlax, Scotland	6.17 ± 0.01	0.961 ± 0.001	66.0 ± 0.1	4.1 ± 0.0	5.9 ± 0.0	NA	NA	NA
2	S. Somon, Gravadlax, Turkey	6.54 ± 0.05	0.959 ± 0.001	66.5 ± 0.6	1.6 ± 0.1	2.4 ± 0.1	3.81 ± 0.03	3.76 ± 0.04	NO
4	Fish paste-1, Tuna, Denmark	5.40 ± 0.08	0.919 ± 0.001	41.2 ± 1.7	3.5 ± 0.0	7.9 ± 0.3	5.56 ± 0.7	4.26 ± 0.8	NO
7	Fish paste-1, Anchovy, Turkey	5.86 ± 0.06	0.845 ± 0.001	42.8 ± 1.3	9.5 ± 0.3	18.1 ± 0.0	2.24 ± 0.09	2.10 ± 0.06	NO
8	Fish paste-2, Anchovy, Turkey	6.12 ± 0.05	0.857 ± 0.001	38.6 ± 0.2	8.9 ± 0.1	18.8 ± 0.1	2.74 ± 0.06	2.25 ± 0.08	2.00 ± 0.04
9	Fish paste, Anchovy & Sardine, Turkey	6.26 ± 0.04	0.814 ± 0.002	42.2 ± 0.4	3.8 ± 0.2	8.3 ± 0.1	NA	NA	NA
10	Fish paste, Anchovy, Greece	7.26 ± 0.04	0.825 ± 0.000	58.7 ± 0.1	12.5 ± 0.2	17.6 ± 0.2	NA	NA	NA
11	Fish paste, Anchovy, Italy	5.91 ± 0.02	0.733 ± 0.002	37.7 ± 0.0	20.1 ± 0.1	34.8 ± 0.0	4.65 ± 0.08	4.26 ± 0.06	NO
15	Fléer, Sweden	6.44 ± 0.68	0.841 ± 0.001	64.1 ± 3.4	10.7 ± 0.8	22.9 ± 0.8	NA	NA	NA
18	Erik den rode, Sweden	7.22 ± 0.24	0.905 ± 0.001	70.9 ± 0.4	5.3 ± 0.1	15.5 ± 0.2	NA	NA	NA
20	Lekmogen, Sweden	7.37 ± 0.12	0.910 ± 0.000	76.1 ± 0.5	4.5 ± 0.3	15.8 ± 0.2	NA	NA	NA
22	Måsens (Sweden)	7.07 ± 0.32	0.904 ± 0.001	74.7 ± 1.7	4.1 ± 0.6	14.1 ± 0.2	NA	NA	NA
24	Kryddersild, Denmark	7.09 ± 0.07	0.843 ± 0.003	65.9 ± 2.5	8.7 ± 0.8	20.3 ± 0.3	NA	NA	NA

All the samples originated from fish-processing companies and they were stored at a refrigerator at 4 °C ± 1

±, SD, NA not analysed due to the limitation in sample amount, NO no observation, TVC total viable counts, HFB Histamine-forming bacteria, THB total halophilic bacteria, WPS water phase salt

^a Sample no represents the same numbers stated at Table 1 (Missing numbers indicates that the sample was not analysed due to sample limitation)

gravadlax and fish paste. On the other hand, higher counts were determined for total viable counts and histamine-forming bacteria at 3.81 and 3.76 log cfu/g for *gravadlax* sample, and in the range of 2.24–5.56, 2.25–4.26 and NO-2.00 log cfu/g for fish paste samples, respectively (Table 8). Kuda et al. [52] observed a wide range of bacteria at 2.0–7.0 log cfu/g in the fermented fish paste products. Kılınç et al. [56] observed 2.5 log cfu/g for sardine patties, and the values increased up to 6.72 log cfu/g during storage of 7 days.

Yongjin et al. [16] reported that some *Micrococcaceae* and lactic acid bacteria also have high amino acid decarboxylase activities and can produce putrescine, histamine and tyramine. They demonstrated low pH values substantially suppressed the activity of amine-positive microorganisms, and therefore, the samples with lower pH did not result in a higher amine yield in the end products. Kimura et al. [54] demonstrated that histamine formation is higher in anaerobic conditions compared with aerobic conditions. They indicate that anaerobiosis is unavoidable during the fermentation of salted and fermented products; other factors should be considered to prevent histamine formation in these products.

Conclusion

The study demonstrated that ~17 % of traditional fish products contained histamine values over the FDA permitted levels, while ~8 % were unacceptable according to EU regulations including smoked fish sample close to 100 mg/kg. Only 3 % were unsuitable for Turkish standards. These results may indicate that traditional products from the European countries may contain low histamine health risk. Most samples exceeding permitted levels of either FDA or EU for histamine attributed to Turkish companies. One of the reasons for this might relate to the higher upper limit given for this amine from Turkish authorities. Second reason might be attributed to the less familiarity of Turkish companies for the processing of these types of traditional fish products since their application goes back more recently in Turkey in comparison with European countries.

No relationship was observed between the levels of histamine and other BAs although a salt content of some products seems to affect the levels of biogenic amines. The levels of histamine and other biogenic amines did not closely relate to species type, since the values had a wide range for the products within the same species applied to the same processing type.

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