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Research Paper

Histamine in Fish Products Randomly Collected in Southern Italy: A 6-Year Study

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ABSTRACT

In total, 4,615 fresh and processed fish samples collected from 2010 to 2015 were analyzed for histamine by ultrahigh-performance liquid chromatography with diode array detection. Histamine levels were detected in 352 (7.6%) samples, with a maximum of 4,110 mg kg^{-1} and mean values of 908.9 ± 1,226.79 and 344.01 ± 451.18 mg kg^{-1} for fresh and processed fish samples, respectively. No histamine levels were found in canned tuna and smoked fish samples in contrast to most of the data reported in the literature. A low percentage (2.79%) of noncompliant samples was found. The highest mean values were found during 2011 and 2015 for fresh and processed fish samples, respectively, showing a significant ($P < 0.05$) difference between the sampling years. The histamine contents found in fresh fish samples were significantly higher ($P < 0.05$) than those of processed samples. Most of the positive samples came from street vendors, suggesting the need to improve inspection measures in these commercial categories to ensure fish product safety.

HIGHLIGHTS

- Fresh and processed fish (4,615) were analyzed for histamine presence in a 6-year study.
- A fast and reliable UHPLC-DAD method was carried out and validated for the purpose.
- A low percentage of noncompliant samples with high levels of histamine were found.
- High histamine levels were found in fresh samples collected from street vendors.

Key words: Biogenic amine; Food safety; Fresh fish; Histamine; Processed fish; Ultra-HPLC with diode array detection

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Scombroid poisoning, also called histamine [2-(1*H*-imidazole-4-yl)ethanamine] fish poisoning, is one of the most common forms of intoxication caused by consumption of fish products. Medical reporting of scombroid intoxication is frequent in Italy, mainly in coastal areas where there is a very high consumption of fish and fish products *(7, 26)*. The symptoms of and treatment for this food poisoning are often associated with seafood allergies *(21)*.

The pathology manifests as obvious symptoms that consist of skin redness, throbbing headache, burning mouth, abdominal cramps, nausea, diarrhea, palpitations, malaise, and rarely hyperthermia or loss of vision. Symptoms usually appear within 10 to 30 min after ingestion of fish and are generally self-limiting *(7, 16, 41)*. Scombroid poisoning is usually associated with high levels of histamine (≥50 mg/100 g) in the spoiled fish. Histamine is a biogenic amine. It is formed in the flesh of fish by decarboxylation of histidine, a reaction catalyzed by histidine decarboxylase, an enzyme that is found in some bacterial species in the genera *Morganella, Klebsiella, Photobacterium, Vibrio,* and others *(19, 22, 34, 36, 37, 42)*.

These microorganisms have an optimal temperature range between 20 and 30°C *(24),* although some histamine-producing bacteria can grow at temperatures below 10°C (e.g., *Vibrio* species) *(33)*. Elevated histamine levels in seafood indicate an interruption of the cooling chain, leading to the deterioration of fish even in the absence of typical signs that would represent the nonedibility of the product; therefore, histamine can be used as a valuable quality marker and indicator of the freshness of fish products. Once produced, histamine tends to remain unchanged in the food, because it is particularly resistant to heat, and it is not destroyed by normal cooking temperatures *(11)*.

High histamine levels can be found in fishes with high free-form histidine concentrations; such fishes include mackerel, sardines, anchovies, tuna, and other species belonging to the families Scombridae, Clupeidae, Engraulidae, Coryfenidae, Pomatomidae, and Scombresosidae *(5)*.

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The European Food Safety Authority Panel on Biological Hazards *(15)* recommended that further studies on the toxicity and associated concentrations of histamine and tyramine in different foods are needed as well studies on the related potentiating effects of putrescine and cadaverine, in particular, concerning food involved in outbreaks and sporadic cases. Member states informed the European Food Safety Authority that findings of certain levels of toxic biogenic amines in fermented food could be of concern and reported a recent increase of biogenic amines content in some fermented foods. The "Commission Regulation (EC) No 2073/2005 of 15 November 2005 on Microbiological Criteria for Foodstuffs" set the limits of histamine at 200 mg kg^{-1} for fresh fishes and at 400 mg kg^{-1} for processed fish products *(15)*.

At present, high-performance liquid chromatography (HPLC) is the reference method that must be used to detect histamine *(3)*, enabling simultaneous and high-sensitivity quantification of histamine in foods and revealing the best method for monitoring and control purposes *(3)*. The literature reports various HPLC methods for histamine level evaluation that require the use of different detectors; however, these methods are based on a derivatization step to improve the detection of the histamine by UV or fluorescence spectroscopic detector. These conditions imply a great waste of time, money, or both. The present work aimed to evaluate the histamine content in fish and fishery products commercialized in Sicily (southern Italy) in the years 2010 to 2015 by the development and validation of an ultra-HPLC diode array detection (UHPLC-DAD) analytical method to evaluate the modes of quality loss and safety concerns in fish products sectors of Mediterranean countries and give exhaustive epidemiological data about this topic.

TABLE 1. *List of the fresh and processed fish samples collected*[a]

	n	*n* from supermarkets	*n* from street vendors
Albacore	8	—[b]	8
Anchovies	247	130	117
Bluefin tuna fillets	762	382	380
Codfish	81	40	41
Codfish fillets	9	9	—
John Dory	10	—	10
Mackerel fillets	245	105	140
Sardines	190	115	75
Swordfish	31	19	12
Tuna eggs	9	9	—
Fresh fish	1,592	809	783
Anchovies in oil	539	300	239
Anchovy paste	153	75	78
Canned tuna	55	20	35
Fish-based sauce	7	—	7
Mackerel in oil	51	40	11
Marinated anchovies	106	60	46
Natural canned tuna	56	16	40
Precooked tuna	99	50	49
Round sardinella in oil	62	62	—
Salted anchovies	358	200	158
Salted sardines	108	54	54
Sardinella in oil	708	400	308
Sardines in oil	175	100	75
Smoked tuna	18	18	—
Smoked herring	9	9	—
Smoked salmon	15	15	—
Smoked swordfish	33	33	—
Swordfish carpaccio	9	9	—
Tuna in oil	430	290	140
Tuna by-products (buzzonaglia)	32	15	17
Processed fish	3,023	1,766	1,257
Total	4,615	2,575	2,040

[a] *n,* number of samples examined.
[b] —, no samples collected.

MATERIALS AND METHODS

Reagents and standards. Histamine dihydrochloride (99%), sodium 1-decanesulfonate, potassium monophosphate, potassium hydrogen phosphate trihydrate, acetonitrile, and perchloric acid were purchased from Sigma-Aldrich (Amsterdam, The Netherlands). All the chemical reagents and solvents were of analytical and chromatographic grade, respectively. Ultrapure water was obtained from a Millipore purification system (Millipore, Burlington, MA).

Phosphate buffer solution at pH 6.9 was prepared by weighing 1.7 g of potassium monophosphate, 2.85 g of potassium hydrogen phosphate trihydrate, and 0.49 g of sodium 1-decanesulfonate dissolved into 1 L of distilled water. The histamine standard aqueous solution at 1,000 mg L^{-1} was prepared with 166 mg of histamine dihydrochloride in 100 mL of deionized water. The solution was stored at 4°C for up to 4 months, according to Muscarella et al. *(31)*. Calibration standards solutions were made at concentrations of 5, 20, 40, 80, and 120 mg L^{-1} by the dilution of 1,000 mg L^{-1} histamine standard solutions with deionized water.

Sampling plan and sample collection. In total, 4,615 fresh and processed fish samples were randomly collected from 2010 to 2015 from supermarkets ($n = 2,575$) and from street vendors ($n = 2,040$) in Sicily by local health services (Table 1). All the fresh and processed fish samples collected belonged to 11 fish species (Table 1): *Engraulis encrasicolus* (anchovies), *Thunnus thynnus* (bluefin tuna), *Xiphias gladius* (swordfish), *Salmo salar* (salmon), *Zeus faber* (John Dory), *Sardina pilchardus* (sardines), *Scomber scombrus* (mackerel), *Coryphaena hippurus* (mahi-mahi), *Sardinella aurita* (sardinella), *Clupea harengus* (herring), and *Thunnus alalunga* (albacore). Each fresh fish sample was transported to the laboratory of the Istituto Zooprofilattico Sperimentale della Sicilia in iceboxes and then stored at –20°C until analysis. Disposable knives were used to remove salt from salted products.

Sample preparation and extraction. Approximately 10 g of each sample was homogenized using a B-400 mixer (Büchi, Flawil, Switzerland). The sample was then put into a 50-mL centrifuge test tube and fortified by adding a known amount of the standard histamine solution at 1,000 mg L^{-1}. Ten milliliters of perchloric acid aqueous solution (6%) was added to the weighted sample, and then the mixture was vortexed for 1 min. Next, 30 mL of deionized water was added in the same centrifuge test tube, and the sample was vortexed again for 1 min. The mixture was centrifuged for 10 min at 3,000 rpm (SL 16 Centrifuge, Thermo Scientific, Waltham, MA), and the supernatant was transferred to a

TABLE 2. *UHPLC-DAD method validation parameters*

Histamine level (mg kg^{-1})	Observed concn (mg kg^{-1})[a]	RSD (%)	Expanded uncertainty (%)	Recovery (%)
100	104 ± 0.9	0.9	16	104
200	200 ± 3	1.4	9.5	100
400	401 ± 2	0.5	5	100

[a] Histamine means ± SD ($n = 10$).

50-mL volumetric flask and filled to the mark with deionized water. The solution was then filtered by syringe on a 0.45-μm microfilter directly into the vials.

UHPLC conditions. Chromatographic separations were carried out with an Agilent 1290 diode array detector with UV/DAD (Agilent Technologies, Santa Clara, CA) equipped with a Supelcosil LC-ABZ column (15 cm, 4.6 mm; inside diameter, 5 mm). Injection volume was 20 μL, flow rate was 1.2 mL/min at room temperature, and the detector wavelength was set at 210 nm. The method involved an isocratic elution using mobile phase A that consisted of phosphate buffer aqueous solution at pH 6.9 and mobile phase B that consisted of acetonitrile (85:15, v/v). A certified blank sample spiked with 200 mg kg^{-1} histamine was added and examined every analytical session as the positive control.

Validation of HPLC-DAD method. The method was validated by an in-house validation protocol according to Unificazione Nazionale Italiana (UNI) Comité Europèen de Normalisation (CEI EN) ISO/IEC 17025 (ISO/IEC 17025:2005 "General Requirements for the Competence of Testing and Calibration Laboratories"), and it has been accredited by Accredia, the Italian national accreditation body. The limit of detection (LOD) and the limit of quantification (LOQ) were calculated by analyzing, under repeatability conditions, 10 independent blank samples spiked with 10 mg kg^{-1} of the target analyte, according to the European "Guidance Document on the Estimation of LOD and LOQ for Measurements in the Field of Contaminants in Feed and Food" (EU Science Hub, European Commission 2016). The relative standard deviation (RSD) was calculated by the following formula:

$$\mathrm{RSD\%} = \frac{\mathrm{SD}}{\mathrm{mean}} \times 100$$

where SD is standard deviation.

The linearity of the method was calculated by the linear regression in triplicate of calibration standards solutions at 5, 20, 40, 80, and 120 mg L^{-1} histamine levels as a function of their concentrations. A correlation coefficient (r^2) higher than 0.999 was considered acceptable.

The recovery and expanded uncertainty parameters were determined by fortifying tuna samples with three histamine spiking levels (100, 200, and 400 mg kg^{-1}) and performing 10 replicates for each validation level.

Data collection and statistical analysis. All the results under the LOD of the method were considered for mean evaluation and statistical analysis as half the LOD value, according to Helsel *(20)*. The data obtained were grouped by year of sampling and sample type (fresh fish versus processed fish samples) for statistical analysis. The assumption of normality of distribution was not met; therefore, a Kruskal-Wallis test was carried out to verify significant differences of the histamine contents between the years of sampling. Furthermore, a Wilcoxon rank sum test was carried out to evaluate significant differences between histamine levels of fresh and processed fish samples. All statistical analyses were performed using R version 3.2.2.

RESULTS

The validation results of the HPLC-DAD method are shown in Table 2. The values of LOD and LOQ obtained were 2.2 and 7.2 mg kg^{-1}, respectively. The recovery values of histamine for all the validation levels were between 100 and 104%, similar to those found in the literature *(3, 35, 39)*. The repeatability test showed a low relative standard deviation (0.5 to 1.4%).

The histamine contents of all the fish samples examined are shown in Table 3. Approximately 7.41% (342 of 4,615) of the samples examined showed histamine content, with mean values of 77.67 ± 431.84 mg kg^{-1} for fresh fish samples and 32.21 ± 168.48 mg kg^{-1} for processed fish products. Salted sardine samples (63 of 108) showed the highest incidence of histamine content, with an average of 145.47 ± 184.30 mg kg^{-1}.

The highest histamine levels were found in fresh fish samples such as tuna fillets and anchovies, with average values of 1,318.60 ± 1,565.12 and 1,225.71 ± 1,006.15 mg kg^{-1}, respectively. Approximately 2.90% (135 of 4,615) of the samples examined showed values above the EC Regulation No 2073/2005 limits, 4.58% (73 of 1,592) and 2.05% (62 of 3,023) for fresh and processed fish samples, respectively. Approximately 80% of all the noncompliant samples came from street vendors (Table 4).

The noncompliant samples ranged between 228.50 and 4,110.00 mg kg^{-1} for fresh fish samples and between 402.5 and 3,050 mg kg^{-1} for processed fish samples.

Fresh fish showed the highest incidence of noncompliant samples. Bluefin tuna fillets accounted for 50% (37 of 73) of the fresh fish noncompliant samples. Approximately 25% of the albacore samples examined exceeded the EC Regulation No 2073/2005 limits, with a maximum of 533.30 mg kg^{-1}.

Among the processed fish samples, anchovies in oil showed the highest number of noncompliant samples (4.6% of the processed fish samples). All the anchovy paste, salted anchovy, and smoked tuna samples reached histamine levels below the EC Regulation No 2073/2005 limit (400 mg kg^{-1}). The histamine contents in salted anchovies (10.04 ± 48.21) were lower than those in anchovies in oil (33.09 ± 169.27). No histamine was detected in marinated anchovies, canned tuna, mackerel fillets, smoked swordfish and salmon, mackerel in oil, precooked tuna, smoked herring, roes of bluefin tuna, and nonscombroid fishes such as swordfish, John Dory, and salmon fillets.

TABLE 3. *Histamine incidence and values of the fresh and processed fish samples examined sorted by years (2010 to 2015) of sampling*[a]

Sample type	2010				2011				2012			
	n	>LOD, *n* (%)	Mean ± SD	Noncompliant, *n* (%)	*n*	>LOD, *n* (%)	Mean ± SD	Noncompliant, *n* (%)	*n*	>LOD, *n* (%)	Mean ± SD	Noncompliant, *n* (%)
Fresh	249	1 (0.38)	32.23 ± 357.64	1 (0.4)	169	47 (27.81)	397.24 ± 978.01	41 (24.26)	254	8 (3.15)	6.22 ± 39.63	3 (1.18)
Albacore	—[b]	—	—	—	—	—	—	—	—	—	—	—
Anchovies	73	—	—	—	38	9 (23.68)	477.66 ± 870.32	9 (23.68)	45	2 (4.44)	3.47 ± 9.59	—
Bluefin tuna fillets	120	1 (0.83)	35.34 ± 375.09	1 (0.83)	95	29 (30.52)	482.08 ± 1152.17	29 (30.52)	71	1 (1.40)	32.65 ± 265.83	1 (1.40)
Codfish	—	—	—	—	—	—	—	—	—	—	—	—
Codfish fillets	—	—	—	—	—	—	—	—	—	—	—	—
John Dory	10	—	—	—	—	—	—	—	—	—	—	—
Mackerel	37	—	—	—	27	6 (22.22)	5.26 ± 9.80	—	93	1 (1.07)	4.44 ± 30.65	1 (1.07)
Sardines	9	—	—	—	9	3 (33.33)	199.07 ± 320.79	3 (33.33)	35	4 (11.42)	26.43 ± 91.65	1 (2.85)
Swordfish	—	—	—	—	—	—	—	—	10	—	—	—
Tuna eggs	—	—	—	—	—	—	—	—	—	—	—	—
Processed	376	9 (2.39)	1.1 ± 3.56E^{-15}	—	363	23 (6.33)	7.38 ± 30.69	—	343	18 (5.24)	30.23 ± 165.23	7 (2.04)
Anchovies in oil	162	9 (5.55)	7.43 ± 27.27	—	180	20 (12.34)	84.225 ± 28.82	—	197	9 (4.56)	44.77 ± 221.50	7 (3.55)
Anchovy paste	18	—	—	—	18	—	—	—	54	9 (11.66)	48.42 ± 110.02	—
Canned tuna	—	—	—	—	9	—	—	—	—	—	—	—
Fish based sauce	—	—	—	—	—	—	—	—	—	—	—	—
Mackerel in oil	—	—	—	—	—	—	—	—	—	—	—	—
Marinated anchovies	10	—	—	—	9	—	—	—	5	—	—	—
Natural tuna	—	—	—	—	—	—	—	—	—	—	—	—
Precooked tuna	9	—	—	—	36	—	—	—	—	—	—	—
Round Sardinella in oil	9	—	—	—	8	—	—	—	—	—	—	—
Salted Anchovies	72	—	—	—	63	3 (4.76)	11.05 ± 53.78	—	45	—	—	—
Salted sardines	9	—	—	—	—	—	—	—	—	—	—	—
Sardinella in oil	—	—	—	—	—	—	—	—	—	—	—	—
Sardines in oil	1	—	—	—	3	—	—	—	9	—	—	—
Smoked herring	—	—	—	—	—	—	—	—	—	—	—	—
Smoked salmon	9	—	—	—	—	—	—	—	—	—	—	—
Smoked swordfish	22	—	—	—	—	—	—	—	—	—	—	—
Smoked tuna	9	—	—	—	—	—	—	—	—	—	—	—
Swordfish carpaccio	—	—	—	—	—	—	—	—	—	—	—	—
Tuna in oil	46	—	—	—	37	—	—	—	28	—	—	—
Waste of tuna processing	—	—	—	—	—	—	—	—	5	—	—	—
Total	625	10 (1.60)	9.52 ± 166.92	1 (0.16)	532	70 (13.16)	132.49 ± 582.70	41 (6.56)	597	25 (4.19)	20.84 ± 131.75	10 (1.67)

TABLE 3. *Continued*

Sample type	2013				2014				2015			
	n	>LOD, *n* (%)	Mean ± SD	Noncompliant, *n* (%)	*n*	>LOD, *n* (%)	Mean ± SD	Noncompliant, *n* (%)	*n*	>LOD, *n* (%)	Mean ± SD	Noncompliant, *n* (%)
Fresh	341	9 (2.64)	40.46 ± 320.45	6 (1.75)	268	36 (13.43)	34.54 ± 124.72	12 (4.47)	311	12 (3.85)	25.02 ± 225.67	10 (3.21)
Albacore	—	—	—	—	8	8 (100)	309.24 ± 119.40	2 (25)		—	—	—
Anchovies	—	—	—	—	54	4 (7.41)	43.12 ± 10.50	—	37	—	—	—
Bluefin tuna fillets	267	7 (2.62)	45.01 ± 355.29	4 (1.49)	84	12 (14.28)	14.62 ± 79.44	1 (1.19)	125	3 (2.4)	39.01 ± 347.42	2 (1.6)
Codfish	—	—	—	—	—	—	—	—	81	—	—	—
Codfish fillets	—	—	—	—	9	—	—	—	—	—	—	—
John Dory	—	—	—	—	—	—	—	—	—	—	—	—
Mackerel	52	—	—	—	27	9 (30)	169.47 ± 286.59	8 (29.62)	9	—	—	—
Sardines	22	2 (9.09)	80.78 ± 259.79	2 (9.09)	67	3 (2.01)	9.41 ± 48.80	1 (1.49)	48	9 (18.75)	59.16 ± 127.71	8 (16.67)
Swordfish	—	—	—	—	19	—	—	—	2	—	—	—
Tuna eggs	—	—	—	—	—	—	—	—	9	—	—	—
Processed	655	78 (11.90)	33.07 ± 103.81	15 (2.29)	722	65 (9.00)	31.04 ± 210.81	26 (3.60)	564	36 (6.38)	48.53 ± 207.98	31 (5.49)
Anchovies in oil												
Anchovy paste	18	—	—	—	27	9 (30)	20.86 ± 29.50	—	18	—	—	—
Canned tuna	—	—	—	—	45	—	—	—	1	—	—	—
Fish based sauce	6	1 (16.68)	3.58 ± 6.08	—	—	—	—	—	1	1 (100)	1710	1 (100)
Mackerel in oil	—	—	—	—	37	—	—	—	14	—	—	—
Marinated anchovies	—	—	—	—	1	—	—	—	81	—	—	—
Natural tuna	—	—	—	—	38	—	—	—	18	—	—	—
Precooked tuna	18	—	—	—	27	—	—	—	9	—	—	—
Round sardinella in oil	27	9 (33.33)	116.32 ± 145.26	2 (7.40)	17	9 (52.94)	38.19 ± 51.10	—	1	—	—	—
Salted anchovies	81	9 (11.11)	24.94 ± 75.30	6 (7.40)	69	—	—	—	28	—	—	—
Salted sardines	63	48 (76.19)	208.44 ± 202.86	13 (20.63)	27	15 (55.5)	46.65 ± 54.01	—	9	—	—	—
Sardinella in oil	222	9 (4.05)	3.8 ± 13.49	—	217	5 (2.30)	2.82 ± 11.39		269	31 (11.52)	88.21 ± 272.24	27 (10.03)
Sardines in oil	92	2 (2.17)	4.85 ± 18.73	—	53	—	—	—	17	4 (23.52)	95.74 ± 212.87	3 (17.64)
Smoked herring	9	—	—	—	—	—	—	—	—	—	—	—
Smoked salmon	—	—	—	—	5	—	—	—	1	—	—	—
Smoked swordfish	—	—	—	—	11	—	—	—	—	—	—	—
Smoked tuna	—	—	—	—	2	1 (50)	51.05 ± 71.06	—	7	—	—	—
Swordfish carpaccio	9	—	—	—	—	—	—	—	—	—	—	—
Tuna in oil	110	—	—	—	128	26 (20.31)	109.36 ± 466.86	4 (3.12)	81	—	—	—
Waste of tuna processing	—	—	—	—	18	9 (50)	251.03 ± 291.05	5 (27.77)	9	—	—	—
Total	996	87 (8.73)	35.75 ± 210.76	21 (2.10)	990	101 (10.20)	31.73 ± 190.09	38 (3.82)	875	48 (5.48)	39.76 ± 213.56	41 (4.72)

[a] >LOD, samples with histamine contents above the limit of detection of the method; noncompliant, samples with histamine levels above the EC Regulation No 2073/2005.
[b] —, not detected.

TABLE 4. *Number and percentage of noncompliant samples according to EC Regulation No 2073/2005*

	Noncompliant samples, *n* (%)	
	Supermarkets	Street vendors
Fresh fish		
Albacore	—[a]	2 (25)
Anchovies	—	9 (3.64)
Bluefin tuna fillets	3 (0.39)	35 (4.59)
Mackerel fillets	1 (0.4)	8 (3.26)
Sardines	10 (5.26)	5 (2.63)
Processed fish		
Anchovies in oil	7 (0.56)	27 (2.16)
Fish-based sauce	—	1 (14.28)
Round sardinella in oil	—	2 (3.22)
Salted sardines	4 (3.70)	9 (8.33)
Sardines in oil	—	3 (2.80)
Tuna in oil	2 (0.46)	2 (0.46)
Tuna by-products (buzzonaglia)	—	5 (15.62)
Total	27	108

[a] —, not detected.

The incidence of histamine in the samples examined over the years exhibited a bimodal trend for both fresh and processed fish samples. The fresh fish samples showed a peak of histamine incidence during 2011 (8.9%) and a second peak during 2014 (7.61%), whereas the processed fish samples showed two peaks during 2011 (4.28%) and 2013 (8.18%; Fig. 1). Regardless, the higher percentage of noncompliant samples was found in samples collected during 2015 (85.41%). The highest histamine mean values were found during 2011 and 2015 for fresh and processed fish samples, respectively. A significant difference ($P < 0.05$) in histamine levels was found between the years of sampling. Furthermore, the histamine contents found in fresh fish samples were significantly higher ($P < 0.05$) than those in processed fish samples for all the years of sampling.

DISCUSSION

The method developed in this work showed good performance through two simple and fast solid-liquid extractions, using only aqueous solutions. Furthermore, the method does not require a chemical derivatization process, necessary in some methods to improve the detection of the histamine by the detectors *(10, 40)*. The use of simple aqueous solutions instead of hazardous organic solvents also permitted for a very cheap and rapid method. To the best of our knowledge, this was the survey with the highest number of fish samples examined for histamine detection. The results showed a lower occurrence of noncompliant samples (∼2.89% of the total) than what was found in the literature *(3, 14, 31)*. However, noncompliant samples reached very high histamine contents, with maximum values of 4,110 mg kg^{-1} for fresh fish and 3,050 mg kg^{-1} for processed fish samples, respectively. Most of the noncompliant samples (108 of 135) were from street vendors where fish products are often kept in

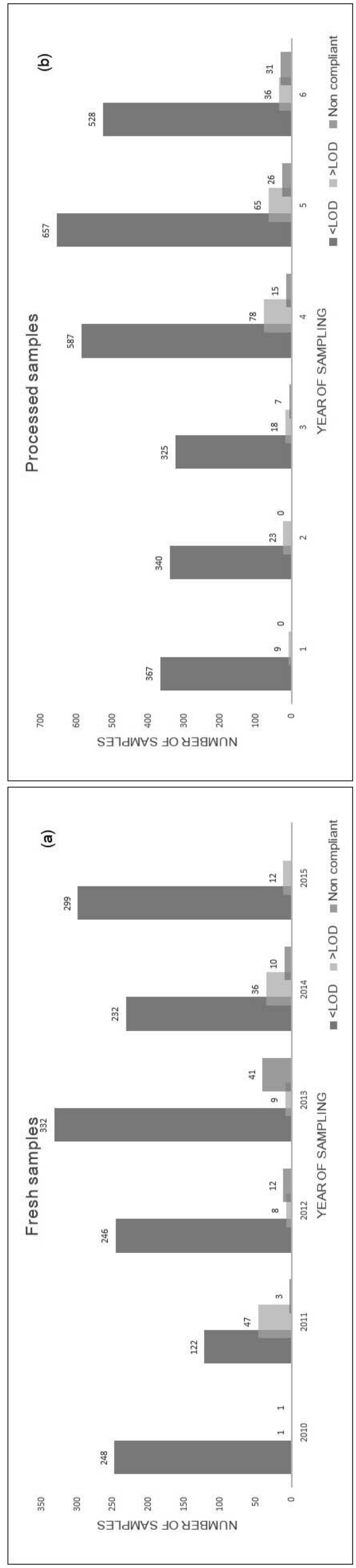

FIGURE 1. *Histamine occurrence of fresh (a) and processed (b) fish samples examined and sorted by year of sampling. Noncompliant represents the number of samples with histamine levels above the EC Regulation No 2073/2005.*

uncontrolled storage conditions. In the majority of cases, fresh fish is displayed on ice; this condition determines a partial covering that lead to an exposition to ambient temperature of the rest *(30, 31)*. Therefore, the high levels reported in our survey suggest bad storage conditions of the fish products examined, leading to microbial growth and histamine production. It is well known that histamine accumulation is most often affected by the combination of time and temperature of storage. Unfortunately, we were not able to have a real estimation of the temperature after the collection and the time and temperature of storage to evaluate a possible correlation with the histamine contents. Only 3.67% of the fresh mackerels examined showed histamine levels above the LOD of the method, but with mean values higher than what was found in Indian mackerels (*Rastrelliger kanagurta*) of Pakistan (144.72 $\pm$ 2.47 mg kg^{-1}) and India (47.73 $\pm$ 2.09 mg kg^{-1}) *(2, 25)* and with Mediterranean mackerels commercialized in Morocco *(1)*. A further comparison with Italian studies revealed mean contents higher than what was found in fresh mackerels collected in Puglia (southern Italy) *(31)* and Bologna (northern Italy) *(28)*. Very high histamine concentrations were also found in 1.69% of fresh and processed anchovies such as anchovy paste, a value much higher than what was reported in the literature *(28, 38)*. Fresh tuna samples showed the highest histamine concentrations, in accordance with most of work reported in the literature *(1, 5, 12, 28),* confirming that tuna is more susceptible to histamine development than mackerel or sardines, because of its high content of free histidine and the composition and levels of bacterial flora *(4, 27)*. The increasing percentage of noncompliant tuna samples during 2014 and 2015 could be related to the individual quotas assigned for the 2014 fishing campaign and imposed by Ministerial Decree 148 of 8 May 2014 *(29)*. This restriction has led many Italian fishers to create a fraud system of hiding tuna specimens in the hold, and selling them at night, on the black market *(23)*. During the storage in the hold, the tuna specimens remaining for a long time in inadequate conditions, leading to an increase of histamine contents. The processed tuna examined in this work revealed the presence of histamine only for tuna in oil samples; of these, only a low percentage (0.93%) of noncompliant samples was found. It should be noted that all these samples were collected from street vendors, most of whom stored the product in nonsterile glass containers without complying with food hygiene standards. However, our levels are much lower than what was reported by Auerswald et al. *(5)* in typically dried tuna preparation of South Africa (Biltong). Contrary to other studies *(13, 38, 42),* no histamine was found in canned tuna, suggesting the good practices carried out by the fish processing industries of the national territory. Furthermore, no histamine was detected in nonscombroid fishes, confirming that these differences can be due to differences on histidine contents *(17)*. However, recent studies showed the presence of histamine in mahi-mahi and swordfish fillets *(8, 9)*. The results on processed fish samples verified a decrease on histamine incidence from 2014, revealing an increasing use of hazard analysis and critical control point systems by the Italian fish processing industry. The results of this work prove that the presence of histamine in seafood remains an issue of concern to food safety, confirming the need for further inspection measures to protect the public health.

The present work aimed at providing an exhaustive overview on the presence of histamine in fish products commercialized in Sicily (southern Italy). A very fast, cheap, and eco-friendly isocratic UHPLC method using a UV-DAD detector has been developed for this purpose. The results showed a low percentage of noncompliant samples but with very high concentrations for fresh and processed fish samples. Analogous studies on the histamine assessment of random samples generally report lower levels but higher percentages *(3, 14, 31, 42)*. Fish is an essential source of protein, vitamins, and minerals, but its nutritional contribution to a balanced diet can be compromised by the high presence of natural contaminants *(6, 18, 32)* and biogenic amines such as histamine. To the best of our knowledge, the present work shows the highest number and variety of seafood samples analyzed to date, providing important data toward a scientific basis for further regulatory policies.

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