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The cold chain and the COVID-19 pandemic: an unusual increase in histamine content in fish samples collected in Southern Italy during lockdown

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Abstract

Objectives: We analysed 900 samples of fresh (250) and processed (650) fish products collected in Sicily (Southern Italy) in 2020 during the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic (hereafter: COVID-19). **Materials and methods:** The samples were divided temporally based on five phases relating to the various restrictions imposed by the Italian government in this period. The validated method of ultra-high performance liquid chromatography (UHPLC) combined with a diode array detector (DAD) was then employed for the analysis. **Results:** The samples collected during the Phase I lockdown period and after it had ended (Phase II) revealed significant increases in the mean histamine levels: 41.89±87.58 mg/kg^{-1} and 24.91±76.76 mg/kg^{-1}, respectively. The 11 (1.3% of the total) fresh fish samples that were identified as being non-compliant with EC Reg. 2073/2005 were only found during these two periods. All the processed samples were always compliant. The histamine values decreased as the restrictions eased, achieving a mean value of 11.16±9.3 mgkg^{-1} (Phase III). **Conclusions:** There was an increase in the incidence of fish samples that were non-compliant with EC Reg. 2073/2005 compared to previous surveillance data. These results provide a first report on the effect of lockdown measures on food safety and the cold chain. Our findings must cause food safety operators to intensify their controls over fresh fish products in such periods to safeguard consumer health. Further studies are required to evaluate whether the same trend would be observed with other food contaminants.

Keywords: histamine; cold-chain; food safety; COVID-19

Introduction

In December 2019, China reported a cluster of cases of pneumonia with an unknown cause that would later be designated as COVID-19. The disease is caused by the acute respiratory syndrome coronavirus-2 (SARS-CoV-2), and has caused significant harm to the global population, including in the form of major socio-economic damage (Chamola et al., 2020). Two months after the first case was reported, the World Health Organization (WHO) declared a public health emergency of international concern (Wu and McGoogan, 2020). The first case of COVID-19 reported in Italy was in January 2020; just one month later, more than 300 cases were recorded (Santacroce et al., 2020; W.H.O, 2020). A number of restrictions were imposed by the Italian government to contain the spread of the disease in the country. A national lockdown was initially put in place from 9 March to 3 May (Conte and Speranza 2020a Mar 9). In this period, known as Phase I, people could only move across regions for health or business purposes. All schools were closed and only grocery stores and shops selling other essential items remained open. During Phase II (4 May to 14 July) and Phase III (15 June– 7 October), there was a gradual loosening of the containment measures in correspondence with the downwards trend of the epidemic up to 5 November, 2020.

After this date, the government introduced dynamic restrictions to contain the infection that were based on 21 parameters such as the number of cases and the capacity of intensive care units to admit patients.

The measures first adopted even included a suspension of the activities of the country's accommodation, catering and travel sectors. This, along with clear changes in the ways consumers ate, shopped and interacted with food, caused significant damage to the fishing industry (Cavallo et al., 2020). Generally, there was an increase in the consumption of comfort and unhealthy food, which was associated with the negative emotions experienced due to the pandemic (Ben Hassen et al., 2020). Furthermore, the rise in online food shopping led to problems obtaining delivery slots (Hobbs, 2020). In the south of Italy, local markets were closed to the general public during phases I and II, leading to a surplus of unsold fish products. The changes put in place because of COVID-19 may have caused less attention to be paid to the health aspects of the food sold during the lockdown (Aday and Aday, 2020), probably due to the extended food storage dates introduced at this time.

Histamine is a biogenic amine (BA) that can be found in fish and fish products. It is produced due to the decarboxylation of histidine, a reaction catalysed by histidine decarboxylase, which is found in some bacterial species belonging to genera including *Morganella, Klebsiella, Photobacterium,* and *Vibrio* (Ababouch et al., 1991; Bjornsdottir et al., 2009; Hwang et al., 2020; Wang et al. 2020). The fish species with high histidine levels belong to the *Scombridae* (*Scomber scombrus*, *Thunnus thynnus*), *Clupeidae* (*Sardina Pilchardus*, *Clupea harengus*) and *Engraulis* (*Engraulis encrasicolus*) (Colombo et al., 2018) families. The histamine levels in fish-based products can rise if they are subjected to poor storage conditions (Lehane and Olley, 2000). Consequently, it can be used as both a valuable marker of quality and an indicator of the freshness of such items (Bodmer et al., 1999; Sánchez-Pérez et al., 2018). Histamine plays numerous roles in the human body, but an increased level in the blood can produce several symptoms through its actions with histamine receptors (Schnedl et al., 2019). Scombroid poisoning is one of the most common forms of intoxication due to the histamine in fish products (Feng et al., 2016). The symptoms of this type of food poisoning are often associated with seafood allergies, and treatment is similarly linked (Hungerford, 2010). Histamine levels in fish products are regulated by European Commission (EC) Regulation No 2073/2005 (European Commission 2005). The maximum levels permitted are 200 mg/kg^{-1} and 400 mg/kg^{-1} for fresh and processed fish products, respectively. Histamine cannot be degraded with standard cooking methods, meaning that prevention measures are crucial (Chung et al., 2017). A cold chain is an essential method for preventing histamine formation. High temperatures can increase its presence in food irreversibly (Hattori and Seifert 2017), and the production of histidine decarboxylase cannot be averted at low temperatures (EFSA, 2015). The goal of the current study was to evaluate whether the restrictions imposed during the COVID-19 pandemic had an impact on the presence of histamine in the fresh and processed fish products sold by supermarkets and street vendors in Southern Italy.

Materials and method

Reagents and standards

Histamine dihydrochloride (99%), acetonitrile, potassium monophosphate, perchloric acid, sodium 1-decanesulfonate and potassium hydrogen phosphate trihydrate were purchased from Sigma-Aldrich (Amsterdam, The Netherlands). All chemicals and solvents were of analytical grade. Ultrapure water used for analysis was obtained from a Millipore purification system (Millipore, Burlington, MA). Standards solutions of 5, 20, 40, 80 and 120 mg L^{-1} were made by diluting a 1.000 mg L^{-1} histamine standard solution (Sigma-Aldrich (Amsterdam, The Netherlands) with deionized water.

Sampling plan and sample collection

In 2020, we collected 900 fish samples (250 fresh and 650 processed) at random from supermarkets and street vendors in Sicily (Southern Italy). All of processed samples were produced in Italy from March to December 2020, as reported on the relevant labels. The samples were grouped for the statistical analysis according to the restrictions introduced by the Italian government due to COVID-19. Four different phases were then identified based on these same restrictions.
Phase I refers to the national lockdown that lasted from 9 March to 3 May and it was during this period that the severest restrictions were in place. Few types of economic activity were permitted (pharmacies, para-pharmacies and grocery stores), public events were forbidden and restaurants were closed. Phase II was subsequently established by the Italian government and covered the date range 4 May to 14 June. Take-away food was available and public parks were open in this period. The downwards trend of the epidemiological curve led to Phase III, during which the government left

only minor restrictions in place. This stage lasted from 15 June to 7 October and allowed public events for up to 200 attendees indoors and the reopening of cultural and social centers. Phase IV (8 October- December 2020) was characterised by the start of a second wave of cases. Consequently, dynamic restrictions were announced for 5 November onwards. The extent of which depend on the evolution of certain epidemiological parameters.

Eighty-six samples were collected during the lockdown (Phase I), 207 in Phase II, 399 in Phase III, and 208 in Phase IV. They belonged to six species: *Thunnus thynnus* (bluefin tuna; n=401); *Sardina pilchardus* (sardines; n=367); *Engraulis encrasicolus* (anchovies; n=62); *Scomber scombrus* (mackerel; n=71); *Coryphaena hippurus* (mahi-mahi; n=9); and *Thunnus alalunga* (albacore; n=2). The data are summarised in Table 1. The fresh fish samples were put on steril bags and transported at +4±1°C in a refrigerated vehicle with a temperature control system. They were then stored at -20±1°C before extraction for the UHPLC analysis, which was conducted on the collection day. Salt and oil in the processed samples were removed before the analysis.

Table 1. List of fresh and processed samples collected divided by pandemic phases. Values indicate the number of samples collected in the specific period.

Sample	Type	Phase I		Phase II		Phase III		Phase IV	
		Street vendors	Supermarket	Street vendors	Supermarket	Street vendors	Supermarket	Street vendors	Supermarket
Fresh									
Anchovies	Whole	-	-	-	-	4	6	4	5
Tuna	Fillets	16	20	28	26	25	25	14	13
Mackerel	Fillets	-	-	-	-	5	4	-	-
Mackerel	Whole	-	-	5	4	-	-	4	15
Sardines	Fillets	-	-	5	4	8	10	-	-
Total	Fresh	16	20	38	34	42	45	22	33
Processed									
Albacore	Marinated	-	2	-	-	-	-	-	-
Anchovies	In oil	2	3	-	-	9	9	-	-
Anchovies	Salted	3	2	-	-	4	5	-	-
Anchovies	Marinated	4	2	-	-	-	-	-	-
Tuna	In oil	3	3	-	-	30	24	38	70
Tuna	Salted	-	8	3	6	-	-	-	27
Tuna	Mixed	-	-	-	-	-	4	-	-
Mackerel	In oil	-	2	-	9	-	9	-	9
Mackerel	Salted	-	3	-	-	-	-	-	-
Mackerel	Marinated	-	2	-	-	-	-	-	-
Mahi-mahi	In oil	-	-	6	3	-	-	-	-
Sardines	In oil	-	-	-	63	55	90	-	9
Sardines	Salted	1	-	23	13	33	30	-	-
Sardines	Marinated	-	4	-	9	-	-	-	-
mixed fishes	In oil	-	-	-	-	3	7	-	-
mixed fishes	surimi	-	6	-	-	-	-	-	-
Total	Processed	13	37	32	103	134	178	38	115
Total		86		207		399		208	

Extraction procedure

The extraction of the samples was carried out according to *Cicero et al.* (Cicero et al. 2020). Briefly, 10 g of samples were homogenized and placed in a 50 ml tube with the addition of 10 ml of perchloric acid aqueous solution (6%). The sample was vortexed for 1 min. Next, thirty milliliters of deionized water were added to the sample and vortexed for 1 min. The solution was centrifuged at 5098 xg for 10 min, then at ambient temperature, the supernatant was transferred to a 50 ml flask and made up to volume with deionized water. A total of 1 ml was filtered on a 0.45 µm microfilter and put on vials for the UHPLC analysis. Each sample were analysed in duplicate.

UHPLC-DAD analysis

The analysis was conducted on an Agilent 1290 UHPLC with UV/DAD detector (Agilent Technologies, Santa Clara, CA, USA). A supelcosil LC-ABZ (15 cm x 4.6 mm, DI 5 mm) was used for the separation. Acetonitrile and an aqueous phosphate buffer solution at pH 6.9 were used as mobile phase (15:85, v/v). The chromatography conditions and instrumental parameters were set according to Cicero et al. (Cicero et al. 2020). Briefly, injection volume was 20 µL, the flow rate was 1.2 mL/min at room temperature and the detector wavelength was set at 210 nm for a runtime of only 6 minutes. The method involved an isocratic elution using a mobile phase A consisted of the phosphate buffer aqueous solution at pH 6.9 and mobile phase B consisted of acetonitrile (85:15, v/v). The method was validated according to the ISO/IEC 17025:2018 (ISO/IEC 17025:2018). The method showed a limit of quantification (LOQ) of 7.2 mg kg^{-1} and a limit of detection (LOD) of 2.2 mg kg^{-1}. The linearity of the method was calculated by linear regression of the areas obtained from the analysis, in triplicate, of histamine calibration standards solutions, accepting a determination coefficient (r^2) > 0.999. The recovery and relative standard deviation (RSD) parameters were determined by spiking blank tuna samples at three concentration levels (100, 200, 400 mg kg^{-1}), performing ten replicates for each level. The results are summarized in table 2.

Table 2. Results of the validation process of the UHPLC-DAD method. ^{a}mean ± SD (n=10).

Histamine level (mg kg^{-1})	Observed concentration^a (mg kg^{-1})	RSD (%)	Recovery (%)
100	104.0 ±0.9	0.9	104
200	200 ± 3	1.4	100
400	401 ±2	0.5	100

Data collection and statistical analysis

All the data were collected and elaborated with R version 4.0.2 (General Public License). Results under the LOQ of the method were considered as the LOQ value (Helsel 2005) for the statistical analysis. The packages used for R analysis and data visualization were: Rcommander (Rcmdr), ggbiplot2 and pplotly (Kabacoff 2011; Sievert 2020). Data were not normally distributed (Shapiro-Wilk Test p-value < 0.05), therefore a Kruskal-Wallis test was carried out to verify differences between sampling periods for each sample type. Post-hoc analysis was performed with Dunn's test (Dunn 1964) and adjusted with the Benjamini-Hochberg method (Benjamini and Hochberg 1995) to determine differences between groups. Only fresh tuna samples revealed the presence of histamine and statistical analysis among different phases was carried out taking only into account these samples.

Results and discussion

Histamine content and general consideration

The results of the analysis are set out in Figure 1 and Table 3. Histamine was detected in 47 fresh tuna samples (5.00%) at levels between 15.07 and 596.69 mg kg^{-1}. About 1.22% of the samples were over the limits imposed by EC Reg. 2073/2005 (200 mg kg^{-1}for fresh fish products), which is comparable to the multiannual studies conducted in Italy (Cicero et al. 2020; Lo Magro et al. 2020). The non-compliant histamine levels were only found in the fresh tuna samples obtained from street vendors. This confirms that there is a high incidence of this BA forming in this type of product due to the high levels of free histidine in their tissues. High amounts of histamine are due to time and/or temperature abuses during handling and storage (Lo Magro et al. 2020). The street vendors in our study were displaying the sampled fresh tuna on ice, but the FDA-recommended visual checks of the condition of ice around a product were not being made, suggesting an absence of time/temperature controls (FDA 2019). Furthermore, as histamine formation can be affected by evisceration, the high concentrations of this BA in our samples suggest that the fresh tuna was stored for a prolonged period without this occurring. In a recent study on eviscerated yellowfin tuna stored at 30°C for 12 hours, the maximum histamine level achieved was 2400 mg kg^{-1} (Benner et al. 2009). Unfortunately, the tuna samples we collected had all been filleted, and so it was not possible to determine the time that had elapsed between evisceration and the fish being offered for sale on the market. In contrast to the fresh tuna, no histamine was found in the samples of processed tuna and other processed fish products, confirming that the Italian fish-processing industries had continued to comply with mandated hazard analyses and critical control-point procedures, even during the lockdown.

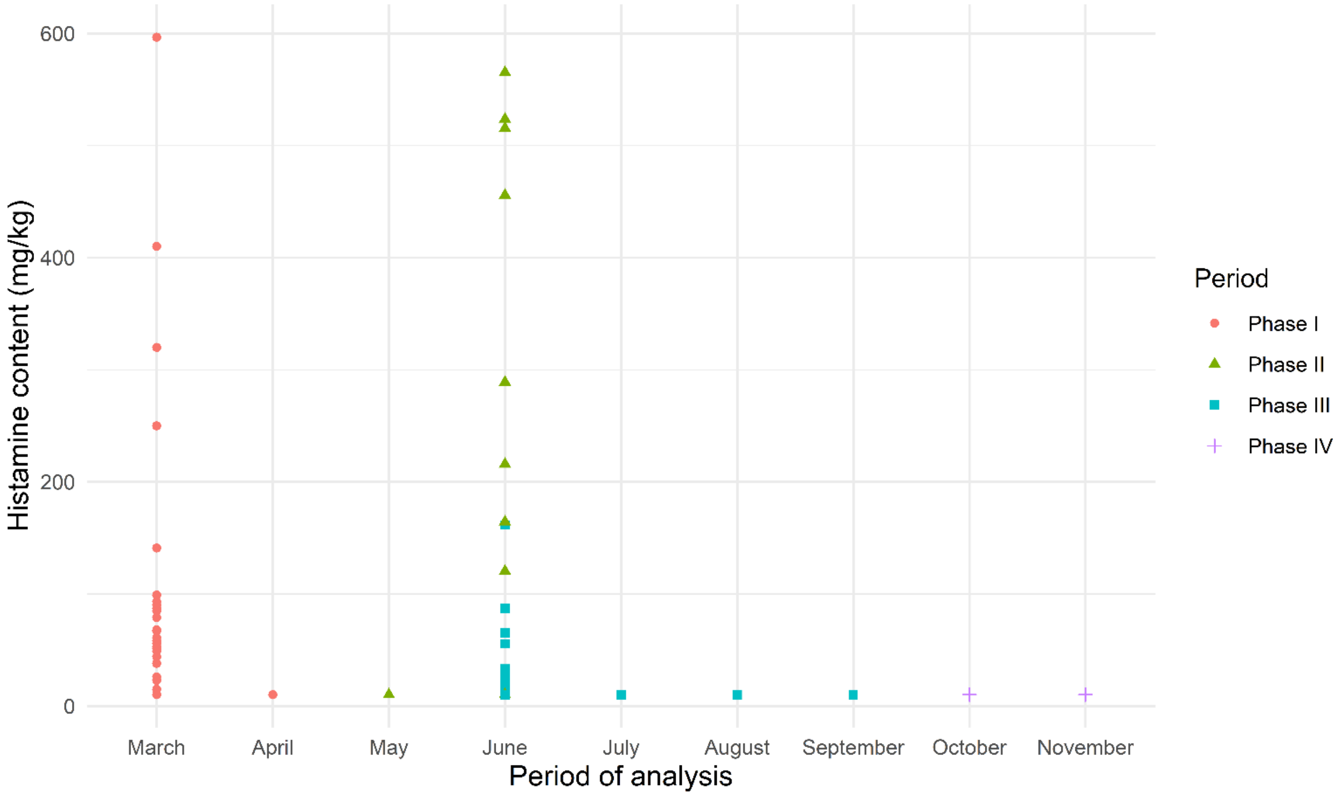

Fig1. Scatterplot of the samples analysed sorted by period and phase of sampling (N = 900).

Table 3. Histamine contents found in the fish samples analysed (expressed as mg kg^{-1}). The observation ">LOD" (Limit of Detection) refers to the number of samples with detectable histamine levels. The term "non-compliant" indicates samples that reached histamine levels above the limits imposed by the EU Regulation (200 mg kg^{-1}for fresh fish, 400 mg kg^{-1} for processed fish), in round bracket the percentage of non-compliant on the total of the sample analysed. The term "analysed" indicates the total number of samples subjected to UHPLC-DAD analysis.

Sample	Phase I	Phase II	Phase III	Phase IV
Fresh				
Analysed	36	72	87	55
>LOD[1]	27	9	8	-
Non-compliant	4 (11.11%)	7 (9.72%)	-	-
Mean ± sd	85.91 ± 123.30	52.51 ±126.15	14.63 ±19.62	-
Processed				
Analysed	50	135	312	135
>LOD[1]	-	-	-	-
Non-compliant	-	-	-	-
Mean ± sd	-	-	-	-
Total				
Analysed	86	207	399	208
>LOD[1]	27	9	8	-
Non-compliant	4 (4.65%)	7(3.38%)	-	-
Mean ± sd	41.89 ± 87.58	24.91± 76.76	11.16± 9.30	-

[1] LOD is 2.2 mg kg^{-1}

Histamine content and pandemic period

Phase I (9 March to 3 May)

The samples from Phase I were the least compliant with EC Reg. 2073/2005, the incidence of non-compliant samples was4.65%. This figure increased to 11.11% if we only considered the fresh samples. Four fresh tuna fillets contained histamine levels above the EC limit, with a concentration range from 250.02 to 596.69 mg/kg^{-1}. All the non-compliant samples were collected between 17 and 31 March 2020 and came from street vendors. The statistical analysis for the fresh tuna revealed significant differences between the first and the other phases examined in the study (Kruskal-Wallis chi-squared = 73.081; $p < 0.05$). There may be a variety of reasons for these results, including in relation to the containment measures imposed during Phase 1 and the radical change in consumer attitudes that occurred in this period (Aday and Aday, 2020). This phase saw panic buying (Hossain, 2020) and a preference for frozen meals (Galanakis et al., 2021). The convenience during lockdown of processed food with extended use-by dates meant that it was favoured over highly perishable items like fresh fish (Cavallo et al., 2020). In addition, the lack of slots available for the delivery of online food orders (Hobbs, 2020) and the consumer's reduced purchasing power also increased the demand for processed food (Khan and Moverley Smith, 2020). As an example, the period 17 February-15 March, 2020 saw the sales of canned tuna increase by +36% compared to the same dates in 2019. Furthermore, Phase I had included a ban on fishing and aquaculture activities until 26 March, 2020 to protect the health of local fisherman, a consequence of which was a reduced supply (Ministero dell' Interno, 2020).

Along with the closure of restaurants, the factors referred to above led to a decline in the demand for fresh fish (D'Oronzio et al. 2020). Consequently, there was initial overproduction during the first lockdown: fresh fish went unsold and fish market operators and street vendors, probably due to liquidity issues, may have decided to keep their fresh products for longer in uncontrolled storage conditions. The result may have been less compliance with permitted histamine levels, as reflected in our samples (Mattioli et al., 2020; Swinnen and McDermott, 2020). Furthermore, flawed mitigation measures agreed by those involved in fishing caused an increase in the amount of unsold fish products (D'Oronzio et al., 2020). Activities began to recover slightly in April 2020, albeit with an ongoing, significant reduction in the fishing effort (in terms of days and hours).

These conditions could explain the absence of non-compliant samples during April despite the first lockdown period. The incidence of non-compliant samples found during the first lockdown period was higher than over 5-years studies conducted in Southern Italy (Muscarella et al. 2013; Piersanti et al. 2014; Cicero et al. 2020), suggesting an alarming situation during this period. Food chain is susceptible to alteration in food demand and offer (Aday and Aday 2020) and food fraud could occur more easily in lockdown due to the fact that there were less food inspections and less governance (Brooks et al. 2021). No histamine was detected in the processed samples, confirming that the fish processing industry in Italy continued to comply with regulations and operate good practices.

Phase II (4 May – 14 June, 2020)

Samples from seven tuna fillets were non-compliant with EU legislation during Phase II (4 May – 14 July, 2020), with a mean histamine content of 407.63 ± 139.78 mg/kg^{-1} and a range between 215.76 mg/kg^{-1} and 565.21mg/kg^{-1}. The percentage of non-compliant samples in Phase II was 9.72% if only the fresh samples were considered. All of these samples were collected from street vendors. Phase II was characterised by less restrictive regulations than Phase I (17 May, 2020).

This slight decrease in the number of non-compliant samples can be linked to both the fact that there was only a gradual loosening of the COVID-19 restrictions and the simultaneous reorganisation of the surveillance procedures on food safety imposed after the national lockdown. Several food fraud cases were brought against street vendors in Sicily. In the majority, fresh fish was displayed on ice, which provides only partial coverage of an item and exposes the rest of it to ambient temperatures. Consequently, the high incidence of non-compliant samples in our study suggests an absence of time/temperature controls of the fish products examined, leading to microbial growth and histamine production.

In this period, restaurants could sell food to take away and via home deliveries. They were able to also serve food at the table from 1 June, 2020, but with limitations in place relating to social distancing. In concert with this, there was a gradual increase in the consumption of fish products.

Phase III-Phase IV (15 June – December, 2020)

No non-compliant samples were found from the period 15 June to December, 2020, despite an increase in the number we collected in Phase III (n=399). June, 2020 onwards saw an increase in the domestic consumption of fresh fish (D'Oronzio et al., 2020; EUMOFA. 2020.). The Hotelier-Restaurant-Café (HORECA) channels were also open in this period, leading to a rise in demand. In Sicily, local restaurants predominantly buy their fresh fish from local and street vendors, who have an important sales channel as a result. The increased demand in this phase was not, however, matched with an adequate supply. This was related to the size of Sicily's fishing vessels, 80% of which are less than 10 m in length, meaning that they could not be used because of the government's social distancing requirements (D'Oronzio et al., 2020). Furthermore, the fear of a production surplus, as occurred in Phase I, caused the sector to reduce its fishing activities, giving rise to conditions that were the complete opposite of those seen in the first lockdown period (EUMOFA, 2020). Phases III, IV and V were therefore characterized by a significant reduction in the availability of fresh fish. Most of the fish caught were sold, and so the sector did not need to adopt inadequate storage practices that could compromise the health of consumers. The restrictions imposed by the government and the closure of the HORECA channels thus seem to have been the key factors undermining the safety of the fresh fish products sold.

Conclusions

This study examines the impact of the restrictions imposed by the Italian government during the COVID-19 pandemic on the safety of fresh fish products. The results reveal a relationship between the extent of these measures and the incidence of samples that did not comply with regulations regarding histamine levels. Furthermore, our findings show that large-scale distributors of processed fish products maintained high hygiene standards compared to street vendors of fresh fish. The latter's non-compliance with safety standards during the lockdown is alarming and should lead to food-safety inspectors intensifying their efforts to ensure that controls on fresh fish products are in place in similar circumstances in the future, using targeted strategies capable of safeguarding health. The limitations of this research are the small data pool and the imbalance of the sample types analysed for each period. Additional studies are therefore required.

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Conflict of Interest

The authors declare no conflict of interest.

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