

Qualitative determination of histamine in canned yellowfin tuna (*Thunnus albacares*) marketed in Sardinia (Italy) by rapid screening methods

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Abstract

Histamine is produced by the bacterial decarboxylation of histidine, an amino acid present in large amount especially in scombroid fish such as tuna. Fish containing high levels of histamine have been associated with many instances of “scombroid poisoning”. Since histamine is heat resistant, its presence has been used as an indicator of the good manufacturing practice and of the preservation state of canned tuna. In this study we have applied a rapid screening method to determine the presence of histamine in canned tuna marketed in Sardinia (Italy). A total of 165 samples of canned tuna were screened for the qualitative determination of histamine by HistaSure™ Fish Rapid Test. The results were consistently in agreement with the food safety criteria (<100 mg/kg of histamine) laid down in EC Regulation 2073/2005 (as amended). The HistaSure™ kit was confirmed as a rapid screening method for the presence of histamine in canned tuna.

Introduction

Histamine is a product of decomposition of the amino-acid histidine caused by the growth of certain Gram – bacteria (e.g. *Morganella*, *Klebsiella*, *Proteus*, *E.coli*, *Hafnia*) in fish tissues of the *Scomberesocidae* and *Scombridae* families, e.g., tuna fish, mackerel, sardine, anchovy (Evangelista *et al.*, 2016). The amount of histamine that forms is a function of bacterial species, temperature and time of exposure, and may exceed 1,000 mg/kg. A histamine intake of 70-1000 mg per single meal may be associated with many instances of a major health problem referred to as “scombroid fish poisoning” (Altieri *et al.*, 2016; Nei *et al.*, 2017). The time of onset of this poisoning ranges from several minutes to 3 h after ingestion of fish containing high

levels of histamine (Silva *et al.*, 2011). Several histamine poisoning outbreaks have been reported in many countries over the years, and it is one of the most prevalent forms of seafood-borne disease throughout the world (Silva *et al.*, 2011; Khan *et al.*, 2017). The “scombroid fish poisoning” generally appears in a slight form with a variety of symptoms including rash, urticaria, nausea, vomiting, diarrhea, flushing, tingling and itching of the skin and evolves in about 8 h but may cause death with the amount of histamine ingested and the individual’s sensitivity to histamine (Silva *et al.*, 2011; Khan *et al.*, 2017). Many incidents go unreported because of the mildness of the disease, lack of required reporting and misdiagnosis (Silva *et al.*, 2011). Histamine was the main bio-contaminant reported by the Rapid Alert System for Food and Feed (RASFF) in the European Union in 2019 (MinSal, 2020). In this period, 25 notifications were related to fish and products thereof mainly from Vietnam (9 notifications) and France (5 notifications). The presence of histamine in fish and fish products has been used as an indicator of the good manufacturing practice and of the preservation state of seafood, for instance canned fish. Incorrect storing conditions induce production and accumulation of histamine even at temperatures as low as 5°C. Koohdar *et al.* (2011) highlighted several food safety issues in the usual fishing method in the Oman Sea and post-fishing procedures used in the local tuna canning industry: 42.2% of the frozen tuna samples showed more than 50 ppm amount of histamine. Moreover, quality loss and histamine accumulation often occur after poor quality frozen fish is thawed and kept for long periods of time at room temperature before further processing (Tsai *et al.*, 2005). At the same time, defective handling techniques of high-quality fish during processing results in the presence of toxic levels of histamine in canned products (Zarei *et al.*, 2011; Mercogliano and Santonicola, 2019). Since histamine is heat resistant, once produced it cannot be destroyed in canned fish products (Khan *et al.*, 2017). Scombrotic fish usually contains levels of histamine more than 200 mg/kg but such fish may be randomly dispersed within a lot. For large fish, histamine is found at variable levels even within individual fish. Sensory evaluation of the fish is not sufficient to detect the absence or presence of histamine; therefore, chemical testing is required (Silva *et al.*, 2011). Quality control measures designed to minimize the occurrence of scombrotic fish require the determination of histamine levels in the range of approximately 10 to 200 mg/kg (Köse *et al.*,

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Key words: Histamine; canned tuna; food safety; screening; rapid methods.

Conflict of interest: The authors declare no conflict of interest.

Contributions: The authors contributed equally.

Funding: This work was funded by FFABR 2017-Fondo per il finanziamento delle attività base di ricerca destinato a finanziare le attività base di ricerca dei professori di seconda fascia e dei ricercatori in servizio a tempo pieno (Legge 11 dicembre 2016 n. 232 (gu n.297 del 21-12-2016 - suppl. ordinario n. 57). CUP J81117000310001.

Ethics approval: This research was conducted in accordance with all relevant guidelines and procedures.

Received for publication: 2 October 2020.
Revision received: 25 January 2021.
Accepted for publication: 22 March 2021.

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Italian Journal of Food Safety 2021; 10:9379
doi:10.4081/ijfs.2021.9379

2011). Good quality fish contains less than 10 mg/kg histamine, a level of 30 mg/kg indicates significant deterioration and 50 mg/kg is evidence of definite decomposition. In the United States of America (USA), the defect action level (DAL), the level at which regulatory actions are taken by Food and Drug Administration (FDA) for histamine, is 50 mg/kg (Bajpai *et al.*, 2020). In the EU, the EC Regulation 2073/2005 (as amended) specified the contents of histamine in fish placed on the market during their shelf-life. In fishery products from fish species associated with a high amount of histidine (particularly fish species of the families: Scombridae, Clupeidae, Engraulidae, Coryfenidae, Pomatomidae, Scomberesocidae), single samples may be taken at retail level. In the nine units comprising the sample, two units may have a value of more than 100 mg/kg but less than 200 mg/kg. No sample unit may have a value ≥ 200 mg/kg. In fishery products which have undergone enzyme maturation treatment in brine,

manufactured from fish species associated with a high amount of histidine and belonging to the above-mentioned families, out of the nine units comprising the sample, two units may have a value of more than 200 mg/kg but less than 400 mg/kg. No sample unit may have a value ≥ 400 mg/kg. On the other hand, the EC Regulation 1019/2013 specified the contents of histamine in fish sauce produced by fermentation of fishery products placed on the market during their shelf-life. The single sample taken at retail level may have a value < 400 mg/kg. Due to the high-resolution power, sensitivity, flexibility, and reproducibility amongst all, HPLC based on liquid chromatography is the analytical reference method in the EU for the analysis of histamine (Nadem *et al.*, 2015). A relatively large number of alternative analytical methods have been reported in the literature for the determination of histamine in such products (Eva *et al.*, 2013; Sarzamin *et al.*, 2017; Tibor *et al.*, 2017; Lili *et al.*, 2017; Khan *et al.*, 2017; Surya *et al.*, 2019). Nevertheless, the major drawback of most of these methods is that they are costly and time-consuming techniques and required high technical skills. Therefore, it is essential to develop sufficiently sensitive and rapid cost-effective methods to detect histamine residues for food diagnosis (Rogers and Staruszkiewicz, 2000). An interest in “portable” procedures for field analysis capable of rapid screening fishery products dockside has led to the development of commercial test kits proposed for Hazard Analysis Critical Control Points (HACCP) plan applications (Köse *et al.*, 2011). The main aim of the work presented herein was the application of one of the simplest and rapid commercial screening procedures (HistaSure™ Fish Rapid Test, LDN, Nordhorn, Germany) for the qualitative detection of histamine in canned yellowfin tuna (*Thunnus Albacares*) samples marketed in Sardinia (Italy). The HistaSure™ Fish Rapid Test (LDN) is a dipstick test intended for the rapid screening of histamine in different scombroid fish types such as tuna, mahi mahi, sardines and for the screening of histamine in fishmeal. The compliance of the Common Organisation of the Markets of Fishery and Aquaculture Products (CMO) rules on the labelling and marketing for preserved tuna (EEC Regulation 1536/92 and EU Regulation 1379/2013) was also evaluated.

Materials and methods

Collection of the samples and evaluation of European Union labelling

From December 2018 to October 2019, a total of 165 samples belonging to the five main Italian canned yellowfin tuna (*Thunnus albacares*) brands (A, B, C, D, E) were collected from large retail stores located in the town of Sassari (Italy). Three cans x80 g of yellowfin tuna (*Thunnus albacares*) in water per month per each brand were included in the study. The samples were transported to the laboratories of the Department of Veterinary Medicine at the University of Sassari (Italy) and were analysed within 24 h of collection. The labels of the five brands were visually evaluated to assess the compliance of the Common Organisation of the Markets of Fishery and Aquaculture Products (CMO) rules on the labelling and marketing for preserved tuna (EEC Regulation 1536/92 and EU Regulation 1379/2013). The main characteristics of the five canned yellowfin tuna (*Thunnus albacares*) labels are reported in Table 1.

Determination of histamine

The presence of histamine in all the samples was carried out by HistaSure™ Fish Rapid Test (LDN). The official protocol provided a cut-off set at 50 ppm Histamine (https://ldn.de/wp-content/uploads/fc-l-3200-en-v13.0_wz.pdf). In case of needs for cut-off adjustments, the manufacturer should be contacted directly to get customized solutions. In order to obtain results easily compared with the food safety criteria laid down in EC Regulation 2073/2005 (as amended), we have decided to set the cut-off to 100 mg/kg histamine and contact the manufacturer for the proper instructions. The cut-off was then adjusted during the sample extraction step by varying the amount of distilled water in which the fish sample was homogenized. All reagents were kept at room temperature (18-25°C) prior to use. The preliminary preparation of the samples for the test procedure was carried out according to the AOAC Official Method 937.07 for canned fish and other canned marine products (AOAC, 2000): the entire content of the can (meat and liquid) was placed in a blender (Koenich, Munich, Germany) and blended until homogenous. Ten g of each prepared canned tuna sample were weighted, added to 490 ml distilled water, and homogenized for 1-2 minutes in a blender (Koenich). The homogenate was then filtered through folded filter paper. An

aliquot of 100 μ l of the filtered homogenate was pipetted into the Acylation Buffer Vials and mixed vigorously by hand. The vials were incubated for 5 minutes at room temperature. 100 μ l of the acylated samples were pipetted into the Running Buffer Vials and mixed gently. The Lateral Flow Device was added to the Running Buffer Vials and incubated for 5 minutes. At the end of incubation, the Lateral Flow Device was removed from the Running Buffer Vial and the results visually read within 5 minutes. A negative control represented by 100 μ l of distilled water was included in each sampling session. The intensity of the test line (amount of immunogold labelled antibody bound to the solid phase histamine and inversely proportional to the histamine concentration in the sample) was compared to the intensity of the control line (upper line) which was always visible. This was the confirmation that the test had operated correctly.

Results and discussion

The visual inspection of the five canned yellowfin tuna (*Thunnus albacares*) labels (Table 1) enabled us to highlight that all the brands followed the CMO-related Regulations rules on mandatory information for the marketing of preserved tuna (EEC Regulation 1536/92 and EU Regulation 1379/2013). In addition to the mandatory information, clear and unambiguous voluntary information were provided by all the Food Business Operators (FBOs). According to the EEC Regulation 1536/92, the trade description of preserved tuna shall be reserved for products prepared exclusively from fish of one of the following species of the genus *Thunnus*: Albacore or longfinned tuna (*Thunnus alalunga*); Yellowfin tuna (*Thunnus albacares*); Bluefin tuna (*Thunnus thynnus*); Bigeye tuna (*Thunnus obesus*) and to the following species of the genus *Katsuwonus*: Skipjack or stripe-bellied tuna (*Katsuwonus pelamis*). All the labels reported *T. albacares* as scientific name and yellowfin tuna as commercial designation. The fishing gear category was not reported in labels A, B and D. Purse seine was reported as fishing method in labels C and E. Yellowfin tuna caught by large-scale purse seiners cannot immediately be collected after entrapping and they remain inside the water for a while with significant duration before being transferred on board to be cooled and subsequently frozen and stored (Koohdar *et*

al., 2010). If these delays are extended, some post-mortem decomposition and accumulation of histamine can occur in these fishes (Yoshinaga and Frank, 1982). Most of the labels always reported two or more fishing areas, most of which were in the Indian and Pacific Oceans. No catch area was reported on the labels of the brand B. The declared conditions for storage and use varied considerably between the five companies: label A recommended to consume the entire content once opened the can. Labels C and D suggest refrigeration of the opened cans and consumption within 1 day. Labels B and E reported only to store the cans in a cold and dry place. The presence of histamine was always <100 mg/kg in all the 165 samples. These results were consistently in agreement with the food safety criteria laid down in EC Regulation 2073/2005 (as amended) for fishery products from fish species associated with a high amount of histidine. Previous studies carried out in canned tuna marketed in several countries showed histamine levels always <100 mg/kg: China (0.02, 19.79, 7.46, and 1.40

mg/kg), Republic of Korea (0.10 mg/kg), Taiwan (45.0 mg/kg), Malaysia (3.20 mg/kg), Iran (77.86 mg/kg), Oman (3.18 mg/kg), Turkey (10.97, 1.38, and 27.05 mg/kg) and Brazil (9.30 and 4.41 mg/kg), (Rahmani *et al.*, 2018). As reported by Mercogliano and Santonicola (2019), high levels of histamine in canned tuna can be also related to the treatment and presentation: grated canned tuna in oil and with tomatoes showed higher levels (9.57 and 17.00 mg/kg, respectively) than grated canned tuna in water and salt (0.36 mg/kg) and solid fish (0.74 mg/kg).

Conclusions

Histamine testing is a possible control strategy that can be used by FBOs to control histamine health hazard in the complex and fragmented food service supply chain, where the main problems are related to the respect of EU framework in terms of food safety and traceability. Restaurants, company canteens,

and cafeterias are the main reported sources of “scombroid fish poisoning” outbreaks (Mercogliano and Santonicola, 2019). The cooking treatment before canning can eliminate both histamine-producing bacteria and their enzymes. Since histamine is heat resistant, once produced, it cannot be destroyed in canned final products because it was present before the heating process started (Khan *et al.*, 2017; Visciano, Schirone and Paparella, 2020). Tuna cans used as ingredient (tuna salad and tuna sandwiches) may be opened hours or week before the preparation or consumption, with likely post-processing contamination and histamine production (Colombo *et al.*, 2018). Although all the companies included in our study were in compliance with the CMO-related Regulations rules on mandatory and voluntary information for the marketing of preserved tuna (EEC Regulation 1536/92 and EU Regulation 1379/2013), the declared conditions for storage and use reported by the companies B and E (only the storage of the cans in a cold and dry place was recommended) should be

Table 1. Main characteristics of the five canned yellowfin tuna (*Thunnus albacares*) brands included in the study.

Brand	A	B	C	D	E
Name of the food	Natural tuna	Natural tuna	Natural tuna	Natural tuna	Natural tuna
Net weight	80g	80g	80g	80g	80g
Drained net weight	56g	56g	56g	56g	56g
Food Business Operator (business name and address)	Yes	Yes	Yes	Yes	Yes
Identification mark	Yes	Yes	Yes	Yes	Yes
Country of origin	Yes	Yes	Yes	Yes	Yes
Tuna species	<i>T. albacares</i>	<i>T. albacares</i>	<i>T. albacares</i>	<i>T. albacares</i>	<i>T. albacares</i>
Catch (FAO) area	34-47-51-71-77-87	n.r.	34-41-47-51-57-71-77-81-87	51-71	34-51-71
Fishing system	n.r.	n.r.	Purse seine	n.r.	Purse seine
List of ingredients	Tuna, water, salt natural celery and onion flavoring	Tuna (85%), water, salt,	Tuna, water, salt, yeast extract	Tuna, water, salt, natural flavors	Tuna, water, salt
Nutrition declaration					
Energy*	97kcal	93 kcal	84 kcal	100 kcal	100 kcal
Fat*	1.0 g	0.5g	0.6g	0.9g	0.9g
Saturated fatty acids*	0.3g	0.2g	0.1g	0.2g	0.2g
Carbohydrates*	0g	0g	0g	0g	0g
Sugars*	0g	0g	0g	0g	0g
Proteins*	21g	22g	20g	23g	23g
Salt*	1.3g	1.1g	1.5g	1.3g	1.3g
Phosphorus	n.r.**	n.r.	162 mg	n.r.	n.r.
Iodine	n.r.	n.r.	50 µg	n.r.	n.r.
Vitamin B12	n.r.	n.r.	2.0 µg	n.r.	n.r.
Best before date/use by date	Yes	Yes	Yes	Yes	Yes
Storage conditions	Keep in a cold and dry place. It is recommended to consume it in its entirety once opened the can	Keep in a cold and dry place	Once opened the can, keep refrigerated and consume within 1 day	Keep in a cold and dry place. After opening, keep refrigerated and consume within 1 day	Keep in a cold and dry place.
Bar code	Yes	Yes	Yes	Yes	Yes

*per 100 g of canned tuna; **not reported.

improved. Since labels help consumers to consciously choose a product according to desirable characteristics (Brom, 2000), FBOs must be appropriately trained on the importance of effective information about the storage and consumption on the labels of their products (Esposito and Meloni, 2017). Commercial test kits based on immunoassay methods for histamine analyses are very popular because of their user-friendliness and reduced time requirements compared to those of traditional analytical techniques (Köse *et al.*, 2011). These simple detection technologies used as screening tests must be reinforced by confirmatory methods if positive results are achieved (Visciano, Schirone and Paparella, 2020). However, previous studies (Köse *et al.*, 2011) reported good agreement of the results obtained by HistaSure™ Fish Rapid Test (LDN) with HPLC results, highlighting that this qualitative kit is suitable for either HACCP monitoring histamine in seafood processing plants or regulatory purposes that uses FDA as well as EU upper permitted limits. HistaSure™ Fish Rapid Test (LDN) offered great advantages and can be easily used as screening kit in HACCP applications.

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