Science Innovation
2014; 2(3): 22-26
Published online July 10, 2014 (http://www.sciencepublishinggroup.com/j/si)
doi: 10.11648/j.si.20140203.11
ISSN: 2328-7861 (Print); ISSN: 2328-787X (Online)

Comparative analysis of histamine levels in commonly consumed frozen fish types in southwest Nigeria

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To cite this article:
Olusina Tunde Ajayi, Folasade Olubunmi Ajasin, Yewande Pat Ajayi, Francis Oladoye Sonibare, Matthew Ojezele, Abraham Oludolapo Oliyide, Samson Olugbenga Ojo. Comparative Analysis of Histamine Levels in Commonly Consumed Frozen Fish Types in Southwest Nigeria. *Science Innovation.* Vol. 2, No. 3, 2014, pp. 22-26. doi: 10.11648/j.si.20140203.11

Abstract: Considering the forms of mishandling observed among fishmongers, it is needful to investigate the histamine content in commonly available frozen fish in Ibadan markets as a predictor of health hazard to consumers. Seventy-two frozen fish samples comprising two (2) different species: Chub Mackerel (*Scomber japonicus*) and Sardine (*Sardinella eba*) purchased from six purposively selected fishmongers each in Bodija and Oja-oba markets were used for this study. Histamine contents and Total Coliform Counts (TCC) were determined. Histamine contents varied with time of the day. Histamine contents of Chub mackerel were higher than the values for Sardine. It may be safer to purchase fish between early hours and midday because the percentage of fish samples that met allowable histamine limit of ≤ 200 mg/kg decreased with time of the day. Also, the percentage of the total fish samples that met the allowable limit was low. This showed poor hygiene practice among the fishmongers as confirmed by the presence of coliform bacteria in all the samples. Since higher percentage of the fish samples in this study was unacceptable for human consumption, there should be routine checking of fish in order to remove implicated products from the market.

Keywords: Analysis, Frozen fish, Histamine, Nigeria

1. Introduction

Fish is of high nutritional benefits because it is rich in protein and essential minerals [1], [2]. It represents part of local and traditional recipes [3]. In Nigeria, frozen fish are extensively consumed [4].

Fish is very heterogeneous and differences may be based on species, handling practice and hygiene [3]. Toxin formation as a result of temperature abuse of fish and poor handling can cause consumer illness [5]. Scombroid fish poisoning is a foodborne illness caused by consumption of scombroid fish such as mackerel as well as non-scombroid fish, such as sardine that contain high levels of free histidine in their muscles [6] – [9]. Histamine is formed by bacterial enzymatic decarboxylation of free histidine in the fish [10], [3]. Gram-positive and Gram-negative bacteria can both produce histidine decarboxylase [11]. Bacterial strains known to be capable of histamine production include *Escherichia, Enterobacter, Pseudomonas, Salmonella, Shigella, Clostridium, Streptococcus, Lactobacillus, Leuconostoc*, *Morganella, Photobacterium, Raoultella, and Hafnia* [12], [11]. According to [13], [9], [14] and [3], the main clinical manifestations affect the skin (rash, oedema and localized inflammation), the gastrointestinal tract (nausea, vomiting, and diarrhea), the haemodynamic (hypotension) and neurological functions (headache, palpitations, tingling, burning, and itching). Histamine may be involved in the onset of migraine attacks in susceptible subjects and may produce hypertensive crises in patients treated with monoamine oxidase inhibitor-type drugs [15],

[16]. In addition to its toxicological properties, histamine is of interest as an indicator of food quality and spoilage [17]. Histamine is also a potent stimulant of both sensory and motor neurons. This stimulation may be important in producing the pain and itching that frequently accompany the urticarial lesions in histamine poisoning [18].

In recent years, the evolving science and debate concerning the benefits and risks of consuming fish have resulted in confusion as to how much, or even if fish should be consumed, and by whom [19]. Thus, attention has been recently increasing, with the aim of ensuring consumer safety [3]. The European Union regulations stipulate that the critical level of histamine is 200 mg/kg or 100 mg/kg according to whether the products have undergone enzyme maturation treatment in brine or not [20]. In the United States, a more stringent level of 50 mg/kg is established [21]. Therefore, the purpose of this study was to determine the contents of histamine in commonly available frozen fish in Ibadan markets as a predictor of health hazard to consumers.

2. Materials and Methods

2.1. Sample Collection

Seventy-two frozen fish samples comprising two (2) different species: Sardine (*Sardinella eba)* and Chub Mackerel (*Scomber japonicus*) were used for this study. A total of thirty-six samples of each species were collected at 8:00, 12:00 and 16:00 hours of the day. They were purchased from six (6) purposively selected fishmongers each in Bodija and Oja-oba markets. The choice of the two (2) sampling locations was informed by their high commercial activities and population densities while the choice of fish species was based on the most consumed by the populace. The samples were collected in sterile polythene bags and transported in ice packs to the laboratory for analysis. Histamine contents and Total Coliform Counts (TCC) were determined.

2.2. Analysis of Samples for Histamine

There are no difficulties in analyzing histamine since a number of suitable methods are available [3]. Histamine content was analyzed spectrofluorimetrically following the procedure of [22]. Four gram (4g) of fish tissue was weighed into a 25ml volumetric flask; it was dissolved in iso-octane. The fluorescence of the compound was measured by means of a Luminescence spectrofluorometer (Perkin Elmer LS 50 B Wellesley, USA), using an excitation wavelength of 350 nm and an emission wavelength of 450 nm. Standard curves were automatically obtained by the spectrofluorometer from known solutions. The results were expressed as mg/kg wet weight of fresh fish muscle. All assays were done in triplicates.

2.3. Analysis of Samples for Total Coliform Counts (Tcc)

One gram (1g) each, of the fish tissue was cut with a sterile knife into 10ml sterile water in test tube, shaken vigorously on a vortex mixer and then serially diluted. From it, 1ml of 10^{-6} dilutions was plated on to MacConkey Agar (McA). The medium was prepared according to manufacturer's instructions. It was sterilized at 121^{o}C for 15 minutes in an autoclave and was poured into sterile disposable petri dishes. The plates were inoculated with 1ml of the diluent at 10^{-6} dilution factor using Pour plate method. The McA was then incubated for 48 hours at 37^{o}C. The bacteria counts were done using colony counter. All assays were done in triplicate and the results are given as mean ± standard deviation. Coliform bacteria were analyzed because they have close relationship with histamine formation in fish.

2.4. Statistical Analysis

The statistical tool/package used was SPSS version 15.0. The data were presented using descriptive statistics. The mean values for histamine were grouped into allowable (≤200) and unallowable (>200). The allowable international standard is 200 mg/kg [3].

3. Results and Discussion

Table I (a). *Relationship Between Histamine Content (Mg/Kg) Of Fish Samples In The Two Markets And Time Of The Day*

Time of the day	HISTAMINE CONTENT (mg/kg) Sardine	
	Bodija	Oja-oba
8:00	223.83±143.30	226.50±165.53
12:00	227.50±138.63	231.00±147.13
16:00	230.80±156.21	235.65±141.56

Table 1 (b). *Relationship between Histamine Content (Mg/Kg) of Fish Samples in the Two Markets and Time of the Day*

Time of the day	HISTAMINE CONTENT (mg/kg) Chub mackerel	
	Bodija	Oja-oba
8:00	257.83±148.92	237.33±114.70
12:00	236.67±129.56	242.00±130.69
16:00	244.17±132.45	260.75±150.20

Table I (a) and (b) showed histamine content profiles of fish samples and time of the day. The histamine contents varied with time except for Chub mackerel from Bodija market which had its highest value at 8:00 hour. This might be attributed partly to the custom of fishmongers in selling the unsold and preserved left-over fish of the previous day in the early hours of the following day. As Table I (a) and (b) revealed, Sardine from Bodija and Oja-oba at 8:00 hour had histamine mean of 223.83±143.30 and 226.50±165.53 respectively while Chub mackerel purchased at the same hour from Bodija and Oja-oba had histamine mean of 257.83±148.92 and 237.33±114.70 respectively. Table I (a) and (b) also showed that the mean values of histamine for Chub mackerel from the two markets were higher than the

values for Sardine from the two markets. It could then be inferred that, Chub mackerel had higher free histidine than Sardine; this was consistent with [5] and [3] assertions that fish species such as mackerel contain high levels of free histidine in their tissues. It could be concluded that histamine production are species-specific because [5] reported that scombrotoxin (histamine) formation of certain species of fish can cause consumer illness. The least value of histamine (58.0 mg/kg) obtained in this study was higher than the result of [23], who reported the histamine content in mackerel to be 6.28 mg/kg. Thus, since histamine accumulation in fish is time-dependent, it is necessary to reduce the time it takes for harvested fish to reach its final consumers in order to reduce to the barest minimum, the incidence of scombroid fish poisoning among fish consumers in the world.

Table II (a). *Relationship between Tcc (Cfu/G) of Fish Samples in the Two Markets and Time of the Day*

Time of the day	TCC (CFU/g) Sardine	
	Bodija	Oja-oba
8:00	$3.67\times10^5\pm1.31\times10^5$	$3.78\times10^5\pm1.27\times10^5$
12:00	$3.81\times10^5\pm1.21\times10^5$	$3.91\times10^5\pm1.65\times10^5$
16:00	$3.98\times10^5\pm1.59\times10^5$	$4.13\times10^5\pm1.60\times10^5$

Table II (b). *Relationship between Tcc (Cfu/G) of Fish Samples in the Two Markets and Time of the Day*

Time of the day	TCC (CFU/g) Sardine	
	Bodija	Oja-oba
8:00	$3.28\times10^5\pm1.54\times10^5$	$3.04\times10^5\pm1.24\times10^5$
12:00	$3.01\times10^5\pm1.16\times10^5$	$3.23\times10^5\pm1.11\times10^5$
16:00	$3.19\times10^5\pm1.07\times10^5$	$3.34\times10^5\pm1.59\times10^5$

Table II (a) and (b) showed the relationship between TCC (CFU/g) of fish samples and time of the day. Coliform bacteria were detected in all the samples analyzed. According to [24], coliforms are not normal flora of bacteria in fish; thus, presence of coliform suggests external contamination. Table II (a) and (b) revealed that contamination increased with time of the day, except for Chub mackerel from Bodija probably due to sales of left-over products. Factors responsible for this increase in contamination levels included poor hygiene in handling and packaging of the fish. These results were similar to the findings of [25]. It can then be concluded that fishmongers have a major responsibility in the prevention of contamination of fish during vending, and if personal hygiene is unsatisfactory, they may cross-contaminate the fish because [11] reported that several foodborne disease outbreaks have been associated with poor personal hygiene of the people handling foodstuffs. According to [3], fish handling is critical with regard to histamine production; since [12] opined that Bacterial strains are capable of histamine production. Thus, [3] added that post-harvest handling, processing and transportation of fish should be given adequate care to ensure quality and consumer safety

Table III. *Histamine Grouping Profiles And Time Of The Day*

Time of the day	Histamine (mg/kg)			
	Allowable (≤ 200 mg/kg)		Unallowable (>200mg/kg)	
	Bodija N (%)	Oja-oba N (%)	Bodija N (%)	Oja-oba N (%)
8:00	5 (41.7)	6 (50.0)	7 (58.3)	6 (50.0)
12:00	6 (50.0)	5 (41.7)	6 (50.0)	7 (58.3)
16:00	4 (33.3)	3 (25.0)	8 (66.7)	9 (75.0)

Table III shows that 41.7% and 50.0% of the fish samples from Bodija and Oja-oba respectively purchased at 8:00 hour met the allowable histamine limit of ≤ 200 mg/kg while only 33.3% and 25.0% of the fish samples at 16:00 hour from Bodija and Oja-oba respectively met the allowable histamine limit. According to [5], in most cases, histamine levels in illness-causing fish have been above 200ppm. From the analysis, it might be safer to purchase fish between early hours and midday since the percentage of fish samples that met allowable limit decreased with time of the day. That means the unacceptability of the fish samples increased as the day progressed from morning to evening hours. This was in agreement with the opinion of [3], that histamine is produced by bacterial actions in fish species which have a naturally high level of free histidine when exposed to a temperature of more than 25° C over a period of more than 6 hours. According to [5[, pathogenic bacteria growth and toxin formation as a result of time and temperature abuse of fish and fishery products can cause consumer illness It can thus be concluded that storage time and temperature are the most critical factors to be controlled to ensure fish consumer safety

Table IV. *Histamine Grouping Profiles and Fish Types*

Fish type	Histamine (mg/kg)			
	Allowable (≤ 200)		Unallowable (>200)	
	Bodija N (%)	Oja-oba N (%)	Bodija N (%)	Oja-oba N (%)
Chub mackerel	7 (38.9)	6 (33.3)	11 (61.1)	12 (66.7)
Sardine	8 (44.4)	8 (44.4)	10 (55.6)	10 (55.6)

AL- Allowable limit; N (%)

Table IV showed that 38.9% and 33.3% of Chub mackerel collected from Bodija and Oja-oba respectively met the allowable histamine limit of ≤ 200 mg/kg while 44.4% of Sardine from both markets met the allowable histamine limit. This low percentage of fish samples from both markets in meeting the allowable limit showed levels of hygiene or handling procedures by the fishmongers in the two markets because according to [3], histamine formation can be easily controlled by applying basic Good Hygiene Practices (GHPs). The levels of coliform contamination observed in this study further confirmed the levels of hygiene in these markets.

4. Conclusion

Therefore, in view of the fact that higher percentage of the fish samples in this study was unacceptable for human consumption, the following are recommended:

Since histamine is heat resistant according to [26], there should be routine checking of fish in order to verify and remove implicated products from the market because [21] opined that seafood containing histamine, above the acceptable standard level should not be used for human consumption and must be subjected to recalls.

Also, regulatory agencies should always organize regular training for fishmongers on food hygiene matters because [19] reported that international and national food safety agencies have recognized the need to provide useful, clear and relevant information to populations that are concerned about making the healthiest choices when considering whether or not to eat fish. Public health education on fish consumption is important since [3] opined that retaining the nutritional value of fish, preserving the benefits of its rich composition and avoiding costly and debilitating effects of fish-borne illnesses are vital.

Acknowledgements

We appreciate Mr Oladapo Muftau Olalekan of Institute of Agricultural Research & Training (IAR&T) Ibadan, for his assistance during laboratory analysis.

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