Freshness and Contamination with Formalin of Mackerel marketed in Dar es Salaam

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Research Article

Keywords:

Posted Date: October 26th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-2194052/v1

Abstract

Background

Fish constitutes a nutritious food that deteriorates quickly when poorly preserved. Several biochemicals, including formaldehyde, naturally accumulate in the fish post-mortem. Apart from this natural formaldehyde, reports indicate the unlawful addition of formalin (37% formaldehyde solution) to the stored fish to prolong freshness**.** This is risky since formaldehyde is carcinogenic, genotoxic, and a potentiator of other carcinogens.

Aim

This study aimed to investigate both the freshness and the extent of contamination with formaldehyde of mackerel sold in Dar es Salaam.

Methods

A total of 60 mackerel samples were conveniently and equally obtained from the local markets, street vendors, and supermarkets in five districts of the Dar es Salaam region. Freshness was evaluated based on organoleptic characteristics. Formaldehyde analysis was done by High-Performance Liquid Chromatography (HPLC). Analysis of variance of formaldehyde concentration in fish flesh by source outlet and district was subsequently run.

Results

The analyzed mackerel samples had acceptable levels of freshness (2.46 ± 0.50) and a mean formaldehyde concentration of 10.89 ± 2.44 mg/kg. On average, the samples from supermarkets were the freshest (2.20 ± 0.21) however the most contaminated with formaldehyde (16.07 ± 4.68 mg/kg), while those from local markets were the least contaminated (3.91 ± 1.86 mg/kg) (p=0.000). Moreover, 0% (n=0), 20% (n=4), and 35% (n=7) of samples from local markets, street vendors, and supermarkets respectively, had formaldehyde concentrations above 20 mg/kg, the previously estimated highest concentration for natural formaldehyde in fish.

Conclusion

Mackerels found in Dar es Salaam have acceptable freshness but are substantially contaminated with formaldehyde. Whether this formaldehyde is natural or artificially added, our findings are inconclusive, given the conflicting global cut-off values for natural formaldehyde in fish. We, therefore, recommend a contextualized study to establish the time dynamics of formaldehyde formation in the stored fish. In the meantime, we advise the public to dwell on the local markets for fish rather than the supermarkets and street vendors.

Introduction

Fish makes food with high nutritive value, being rich in protein, fat, minerals, and vitamins. Specifically, fish contains omega-3 and – 6 fatty acids which are very essential to human nutrition (Hoitsy et al. 2012). If not well preserved, harvested fish quickly deteriorates through microbial and biochemical processes (Ghaly et al. 2010; Islam et al. 2015). For long-term preservation; deep freezing, salting, smoking, and drying are the most preferred methods (Ghaly et al. 2010; Hoitsy et al. 2012).

Consumption of spoiled fish may cause scombroid fish poisoning resulting from the formed scombrotoxins, and is commonest in fish from the families Scombridae and Scomberesocidae (Tortorella et al. 2014; Visciano et al. 2014). Apart from the scombrotoxins, another toxic metabolite formed is formaldehyde. It is formed through the biochemical reduction of an osmoregulatory molecule, trimethylamine oxide (TMAO), which is most prevalent in saltwater fish (Etienne 2015; Ghaly et al. 2010). The concentration of formed formaldehyde is dependent upon the fish species as well as storage temperature, moisture content, and duration (Bhowmik et al. 2017; Etienne 2015; Noordiana et al. 2011).

Apart from its acute effects such as irritation upon ingestion, formaldehyde is a known carcinogen upon prolonged exposure (WHO 2001; WHO IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 2006). Exposure to formaldehyde is reported to have a causal relationship with nasopharyngeal cancer and myeloid leukemia. Moreover, animal studies have revealed the genotoxic potential of formaldehyde and its potentiating effect on other carcinogens. The carcinogenic effects of formaldehyde are stated to be peak exposure dose-dependent. (American Cancer Society 2014; Hauptmann et al. 2003; Pinkerton et al. 2004; WHO IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 2006).

To what extent will formaldehyde naturally form in a particular fish, is presently not a subject of the global agreement due to conflicting suggestions. For example, in Malaysia, it should not be more than 5 mg/kg of fish flesh (Noordiana et al. 2011), while in Italy, it should not exceed 60 mg/kg for Gadidae and 10 mg/kg for crustaceans (Bianchi et al. 2007). In 2001, the World Health Organization (WHO) estimated this formaldehyde to be up to 20 mg/kg for fish and meat (WHO IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 2006). Moreover, in 2014, the European Food Safety Authority (EFSA) proposed the highest concentration of 293 mg/kg for marine products (Etienne 2015).

On its repute, formaldehyde is inherently biocidal, with a long history of use in disinfection and preservative functions (WHO IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 2006). To prolong shelf lives, a 37–50% formaldehyde solution in water, (commonly known as formalin) is deliberately added to fish and other perishable foods by unscrupulous traders in some areas (Islam et al. 2015; Paul et al. 2014; Saba et al. 2015). This is attributed to the increasing access to formalin by the community and its attributes of low cost and high preservative efficacy (BBC News Swahili 2018; Business Focus 2018; WHO IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 2006).

Tanzania, like other developing countries, is facing a rising number of cancer cases, among other non-communicable diseases (IARC 2019; MoHSW-Tanzania 2013). The increasing sedentary lifestyles are partly ascribed to the observed trend (MoHSW-Tanzania 2013). The use of chemicals, particularly in small-scale mining activities (Merket 2018; Purefoy 2013), as well as in the unapproved preservation of perishable foods can be an important precipitator of developing cancer (BBC News Swahili 2018; Ssali 2018; The Citizen 2019;

Wako 2019). Apart from the ongoing efforts by the Tanzania Bureau of Standards (TBS) to explore and tackle these challenges, there are currently no established limits for the quantity of natural formaldehyde in fish consumed in Tanzania.

Along the coastal strip of Tanzania, mackerel is relatively cheaper, readily available, and the most consumed fish type. Like many other fish types from the Scombridae and Scomberesocidae families, mackerel is very perishable at tropical temperatures and demands proper storage (Towers 2015). In Dar es Salaam, the most populated coastal city in Tanzania, mackerel is obtained from local markets, supermarkets, and street vendors who supply door-to-door on foot or bicycles.

Considering the high temperatures along coastal Tanzania (18.1–32.4 ˚C) (WMO 2020), and the ongoing speculations that unscrupulous traders use formalin and other chemicals to preserve perishable food products, we investigated the extent of contamination with formaldehyde of mackerel consumed in the Dar es Salaam city.

Methods

Sampling and sample collection

A total of 60 fish samples (three fish per sample) were conveniently bought from the five administrative districts of Dar es Salaam (Temeke, Ubungo, Kigamboni, Ilala, and Kinondoni). A simulated buyer approach was used to purchase the samples from three sources (supermarkets, local markets, and street vendors) within each district. Four samples were independently collected from each source at an interval of five days. Moreover, one control sample consisting of freshly harvested mackerel was purchased directly from the seashore and used as a blank for the natural matrix setting upon analysis.

Determination of freshness by organoleptic characteristics

An adopted, subjective method that evaluates the freshness-defining organoleptic characteristics of fish was used (Howgate 2011; Patterson et al. 2014). The evaluated characteristics included the odor of the neck, odor, and color of the gills, the general appearance of the fish, slime on the skin and eyes as well as texture of the fish. At least two blinded observers (with no special training) were required to score a given sample for each parameter on a scale of 1 to 5, where 1 was the best score. The overall grade of freshness was obtained as the mean of the scores for each parameter. The freshness was established as excellent (1<2), acceptable/good (2<5), and rejected (5).

Detection and quantification of formalin

Reagents, chemicals, and solvents.

These included; formalin (Merck, Germany), 2,4-dinitrophenylhydrazine (DNPH) (Carlo Erba Reagent group, Spain), Acetonitrile (Sigma Aldrich, USA), and double-distilled water.

Chromatographic conditions

High-Performance Liquid Chromatography (Merck Hitachi Model D- 70001F, Japan) (HPLC) analyses were carried out on an extended C-18 (150 mm x 2.1 mm, 1.5 µm) column (YMC, Japan) as a stationary phase and a mixture of acetonitrile and water (60:40 %v/v) as the mobile phase at isocratic conditions. The mobile phase flow rate of 1 mL/min was used and detection was done at 365 nm using an ultraviolet (UV) diode array detector. Moreover, the samples were injected at a volume of 20 µL and the column oven temperature was set at 40 ºC.

Preparation of the derivatizing agent

2,4-dinitrophenyl hydrazine (DNPH) (**1**) was used to derivatize formaldehyde to enable its detection and quantification in the UV region. In this regard, formaldehyde was converted into a UV- active hydrazone. The derivatizing solution was prepared by dissolving 1.5 g of DNPH crystals in 50 mL of a 20% sulphuric acid solution. In every occasion, this solution was freshly prepared and immediately used (Bhowmik et al. 2017).

Fish sample preparation

For each sample, the skins, fins, and bones were removed from the flesh of the three fish by using a scalpel. The resulting fillets were minced, blended, and homogenized together in an electric blender (Europe strong ES2255, Germany). Five grams of the homogenized flesh were weighed using an analytical balance (Mettler Toledo ML204, Switzerland), and put in a 50mls conical flask, followed by the addition of 5 mL of distilled water. The flask was then capped and sonicated for 40 minutes at 20 ºC followed by centrifugation (HERMLE Labortechnik Z206A, Germany) at 7000 rpm for 10 minutes.

The resulting supernatant was filtered (Whatman no. 1) before drawing 2 mL of the filtrate into another 50 mL conical flask into which, 1 mL of the freshly prepared DNPH solution was added. The flask was thereafter left in dark for 6 hours at room temperature to allow the formation of orange hydrazone precipitate (**2**). The precipitate was captured using a membrane filter (0.45 µm) and dissolved in 2 mL of acetonitrile. The formed solution was re-filtered through a similar membrane filter to remove any undissolved particles before HPLC injections (Bhowmik et al. 2017; Yeh et al. 2013).

Method validation

The adopted analytical method (Bhowmik et al. 2017), was partially validated for linearity, accuracy, the limit of detection, the limit of quantification, as well as precision. Validation was guided by the bioanalytical method validation protocol by the United States Food and Drug Administration (FDA) (FDA 2018) as follows.

Linearity

Preparation of standard calibration curve.

Linearity was evaluated by using a matrix-based calibration standard. This was established by preparing seven known concentrations of formaldehyde spiked on the homogenized control fish sample. Seven concentrations were obtained from a prepared stock solution of 400 mg/L formaldehyde. The range of

concentrations included the Lower limit of quantification (LLOQ) and the upper limit of quantification (ULOQ). Three injections were made at each concentration. For acceptance, the coefficient of variation (CV) of at least four concentrations should have not deviated by more than 15% except at the LLOQ which should have not deviated by more than 20%.

Precision

Inter –Day precision was carried out using six determinations per concentration. Three concentrations of Quality Control samples (QCs) were used and included the lowest concentration which was three times the LLOQ (7.5 mg/L). The others were the mid-range concentration which corresponded to 50% of the ULOQ (45 mg/L) and the highest concentration which corresponded to 75% of the ULOQ (68 mg/L). The acceptance criterion was the coefficient of variation not to exceed 15% at each concentration.

Accuracy

The accuracy of the method was established by determining the mean recovery of three spiked concentrations of formaldehyde in a homogenized control sample. Similar concentration levels were used for the precision above, and six determinations were carried out per concentration. For acceptance, the mean value had to be within 15% of the nominal value.

Limit of detection and limit of quantification

From the matrix-based calibration curve, the slope and peak area was used to obtain the limit of detection and the limit of quantitation.

Statistical Analyses

Analysis of Variance (ANOVA) of formaldehyde in mackerel based on the sources, followed by Tukey's Honest Significance test, were computed using the Statistical Package for Social Sciences (IBM SPSS Statistics 20).

Results

Degree of freshness

All mackerel samples exhibited acceptable levels of freshness (2.46 ± 0.50) (Table 1). Specifically, the samples obtained from supermarkets had the highest levels of freshness (2.20 ± 0.21) whereas those from the street vendors had the lowest levels of freshness (2.83 ± 0.86). However, the perceived differences were not statistically significant ($p = 0.055$).

Table 1
Degree of the freshness of fish samples from different types of vendors.

Districts	Local markets	Street Vendors	Supermarkets	Average	Acceptance	P-value
Temeke	2.42 ± 0.62	2.57 ± 0.57	2.43 ± 0.67	2.47 ± 0.08	Acceptable	**0.521**
Kinondoni	2.25 ± 0.48	2.80 ± 0.70	2.40 ± 0.44	2.48 ± 0.28	Acceptable	
Ubungo	2.29 ± 0.18	2.45 ± 0.05	1.99 ± 0.43	2.24 ± 0.23	Acceptable	
Kigamboni	2.30 ± 0.10	3.87 ± 0.71	2.03 ± 0.15	2.73 ± 0.99	Acceptable	
Ilala	2.13 ± 0.85	2.48 ± 0.34	2.13 ± 0.06	2.25 ± 0.20	Acceptable	
Average	2.28 ± 0.10	2.83 ± 0.86	2.20 ± 0.21	**2.46 ± 0.50**		
Acceptance	Acceptable	Acceptable	Acceptable			
P-value	**0.055**					

Analytical method validation

The analytical adopted method passed all the validation parameters as per the United States Food and Drug Administration (FDA) guidelines (FDA 2018) (Table 2) and (Fig. 1).

Table 2
Method validation parameters

Parameter	Acceptance criteria	Results
Linearity	CV < 15%	1.11–9.75%
Precision	CV < 15%	0.71–4.13%
Accuracy	Mean value 85% – 115%	94.33% – 100%
Limit of Detection (LoD)		0.003 mg/kg
Limit of Quantification (LoQ)		0.01 mg/kg

Detection and quantification of formaldehyde in mackerel

The analyzed mackerel samples were contaminated with formaldehyde at an average concentration of 10.89 ± 2.44 mg/kg and within the range of 0.71–46.01 mg/kg of fish flesh. With respect to the types of vendors, the samples from supermarkets were the most contaminated (16.07 ± 4.68 mg/kg), whereas those from the local markets were the least contaminated (3.91 ± 1.86 mg/g) (Fig. 2). Based on source districts, fish samples from Ilala and Kigamboni respectively, were the most (13.84 ± 9.64 mg/kg) and least (7.13 ± 4.65 mg/kg) contaminated.

Considering the previous WHO's estimation for the highest concentration of formaldehyde in fish flesh (20mg/kg), it was found that 0% (n = 0), 20% (n = 4), and 35% (n = 7) of samples from local markets, street vendors, and supermarkets respectively, had formaldehyde concentrations above that concentration.

Additionally, Ilala and Kigamboni districts had the highest, 33.3% (4/12) and lowest 8.3% (1/12) proportions of samples containing formaldehyde concentration beyond the limit (Table 3).

Table 3
The concentration of formaldehyde in fish samples from three types of vendors across five districts of the Dar es Salaam region

Dar es Salaam Districts	Local Markets	Street Vendors	Supermarkets	Average (mg/kg)	P-Value	Range (mg/kg)	Formaldehyde > 20mg/kg
Temeke	4.91 ± 4.27	7.35 ± 5.06	19.25 ± 10.50	10.50 ± 7.67	**0.244**	1.30 ± 29.12	2 (16.7%)
Kinondoni	3.19 ± 2.05	11.76 ± 5.04	19.24 ± 5.00	11.40 ± 8.03		1.41 ± 23.07	2 (16.7%)
Ubungo	6.65 ± 6.15	12.94 ± 5.74	15.18 ± 5.89	11.59 ± 4.42		1.66 ± 20.80	2 (16.7%)
Kigamboni	2.03 ± 0.93	11.12 ± 7.00	8.25 ± 2.75	7.13 ± 4.65		0.71 ± 20.22	1 (8.3%)
Ilala	2.77 ± 0.47	20.32 ± 16.44	18.44 ± 3.92	13.84 ± 9.64		2.10 ± 46.01	4 (33.3%)
Average (mg/kg)	3.91 ± 1.86	12.70 ± 4.75	16.07 ± 4.68	**10.89 ± 2.44**			
P-Value	**0.000**						
Formaldehyde > 20mg/kg	0 (0%)	4 (20%)	7 (35%)				**11(18.3%)**
Range (mg/kg)	0.71–15.37	1.59–46.01	5.89–29.12			**0.71–46.01**	

Discussion

The freshness of mackerel consumed in Dar es Salaam

This study reports an acceptable degree of freshness of mackerel found in the Dar es Salaam markets. Despite the lack of statistical justification, we report a high level of freshness for fish obtained from supermarkets compared to those obtained from the local markets and street vendors. This can be ascribed to the availability of good freezing facilities (Botelho et al. 2013; Mylona et al. 2017; Sawalha and Palm 2003; Wei and Sammalisto 2011) and probably the speculated unlawful preservation means in supermarkets (The Citizen 2019; Wako 2019).

Compared to those from the supermarkets, samples obtained from the local markets had a lower, however acceptable freshness. That can be linked to the shorter storage periods of fish in those markets caused by high turnover rates. It is from those markets the Dar es Salaam majority and low-income citizens obtain their food products at affordable prices which assure quick turnovers of the sold items. In addition to that, fish in

the local markets is at least sold under shade and some vendors use purchased ice blocks to cool them, hence keeping their freshness appreciably (*personal observation*).

On the other side, samples obtained from the street vendors were the least fresh, and this can be related to the lack of proper storage means and the unhygienic handling of fish by the mobile vendors (*personal observation*). The end result is the exposure of fish to high Dar es Salaam temperatures (WMO 2020), and microbes, among other contaminants that accelerate the loss of freshness and accumulation of biochemical products in fish (Towers 2015).

The extent of contamination with formaldehyde of Mackerel consumed in Dar es Salaam

Our findings reveal substantial contamination with formaldehyde of mackerel consumed in Dar es Salaam, with 18.3% (11/60) of the samples having formaldehyde concentrations above the WHO estimated upper limit of 20 mg/kg for the naturally formed formaldehyde in fish flesh (WHO IARC Working Group on the Evaluation of Carcinogenic Risks to Humans 2006). We further reveal the highest contamination to be associated with samples from supermarkets (16.07 ± 4.68 mg/kg), at a high likelihood (35%) for such samples to be contaminated above the estimated limit.

On the other side, we reveal the lowest contamination (3.91 ± 1.86 mg/kg) to be associated with samples from the local markets without any likelihood (0%) for such samples to be contaminated above the estimated limit. This difference can be ascribed to the high turnover rates in the local markets as described above compared to the prolonged storage of fish in supermarkets. Moreover, the high contamination of supermarket-obtained samples may as well be related to the speculated deliberate addition of formaldehyde among other harmful chemicals, as preservatives to the stored fish (The Citizen 2019; Wako 2019).

The levels of fish contamination with formaldehyde observed in our study are relatively higher than those reported in Ghana and Malaysia whereby formaldehyde was detected in several fish species in the ranges of 0.174 to 3.71 mg/kg and 0.38 to 15.75mg/kg respectively (Asare-Donkor et al. 2018; Noordiana et al. 2011). However, our findings are relatable to those observed in Bangladesh, whereby formaldehyde was detected in marine fish sold in wet markets at a range of 9.08 to 47.55 mg/ml (Bhowmik et al. 2017).

Furthermore, the role played in fish deterioration by the time interval between fish catching and arrival at the selling or storage points was revealed in this study. Compared to the other Dar es Salaam districts, Kigamboni is almost surrounded by the Indian Ocean-the main source of mackerel in the Dar es Salaam markets. Such proximity minimizes the mentioned time interval above, and this can be a cause of the averagely lowest formaldehyde concentration (7.13 ± 4.65 mg/kg) observed in the district as compared to the levels of formaldehyde in the other districts which were above 10 mg/kg.

Conclusion

All tested mackerel samples were of acceptable freshness and those obtained from the supermarkets were the freshest while those from the street vendors were the least fresh. The analyzed samples were variably contaminated with formaldehyde whereby, those from the supermarkets and local markets were the most

and least contaminated respectively. With the current disagreement on the levels of naturally formed formaldehyde in fish, it is impossible to discriminate whether the detected formaldehyde was natural or artificially added in the analyzed fish samples.

Therefore, we recommend a well-controlled and contextualized study aiming to establish the time dynamics of formaldehyde formation in stored fish species. In this regard, freshly harvested fish samples should be followed for selected periods in specified storage temperatures to establish the concentration of the naturally formed formaldehyde per time per temperature. In the meantime, we advise the general public to source fish more from the local markets rather than the supermarkets and street vendors since the samples from the local markets appear to be the least contaminated with formaldehyde and have an acceptable degree of freshness.

Abbreviations

HPLC: High-Performance Liquid Chromatography, WHO: World Health Organization, TMAO: trimethylamine oxide, EFSA: European Food Safety Authority, TBS: Tanzanian Bureau of Standards, IARC: International Agency for Research in Cancer, DNPH: 2,4-dinitrophenylhydrazine, UV: ultraviolet, FDA: United States Food and Drug Administration, LLOQ: Lower limit of quantification, ULOQ: upper limit of quantification, CV: coefficient of variation, QCs: Quality Control samples, ANOVA: Analysis of Variance

Declarations

Ethical approval

This study was approved by the institutional review board (IRB) of the Muhimbili University of Health and Allied sciences.

Authors' contributions

All authors contributed significantly to the development of this manuscript. VM conceptualized and supervised the study. MAU collected data and drafted the study report. RS guided and supervised the laboratory activities. PMM and IJD drafted the manuscript. NEM, NN, and JS reviewed the manuscript. DHS and EK supervised the study and approved the final manuscript.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Availability of Data

The datasets supporting the conclusion of this article are available from the corresponding author upon request.

Funding

Not Applicable

Acknowledgments

The authors convey gratitude to the staff at the Pharm R and D lab of Muhimbili University of Health and Allied Sciences, for their backbone role played in designing and conducting this study.

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Schemes

Schemes 1-2 are available in the Supplementary Files section.

Figures

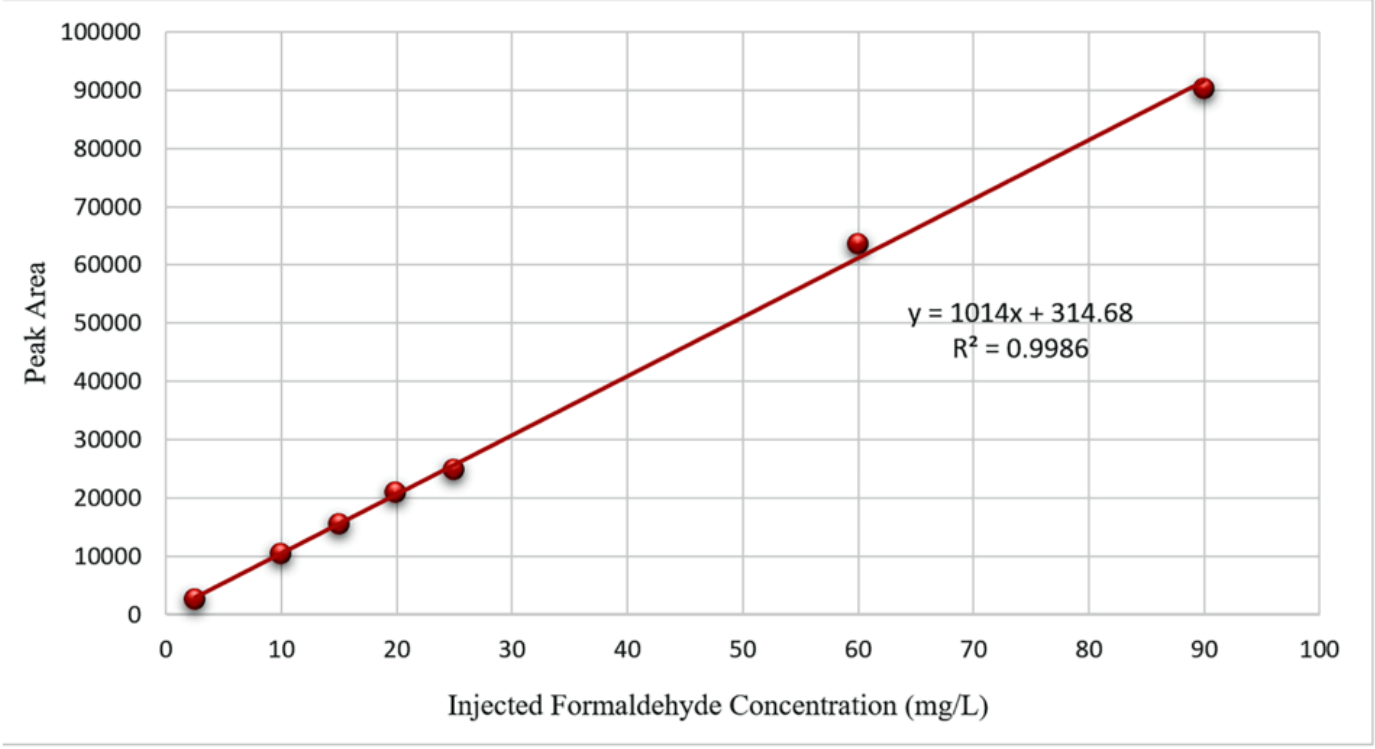

Figure 1

Calibration curve for formaldehyde spiked on the control fish sample.

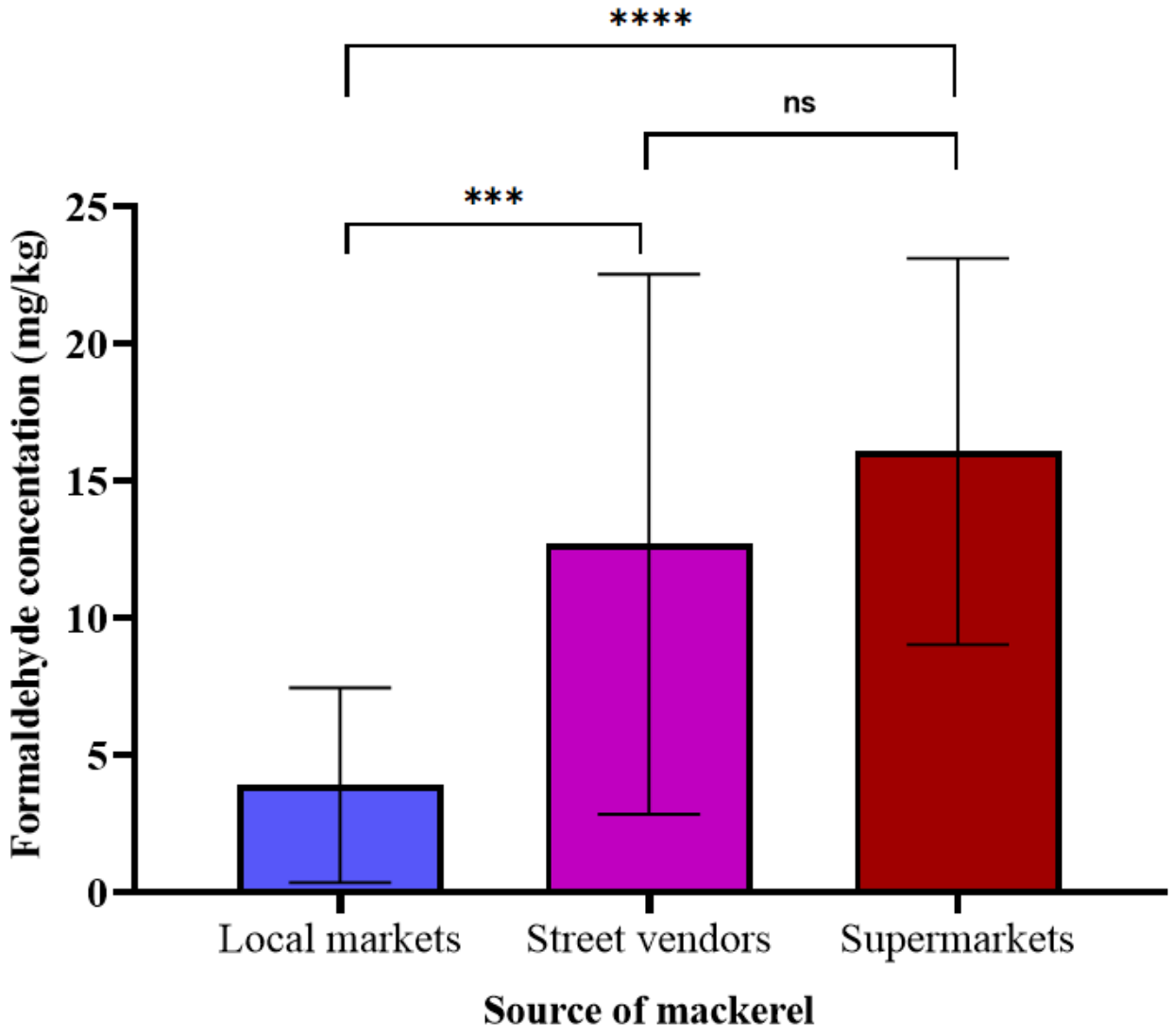

Figure 2

Comparison of formaldehyde contamination of mackerel fish from different outlets in Dar es Salaam in mg/kg of fish flesh. ******** means the difference is significant at p value = 0.0001, ******* means the difference is significant at p value = 0.001, and **ns** means the difference is non-significant.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Additionalfile1.Methodsandresults.docx
- Additionalfile2.DataSummaryandStatisticalanalysis.xlsx
- Additionalfile3.Calibrationcurve.xlsx
- Scheme1.png

- Scheme2.png