Food Safety and Risk

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Organoleptic quality and formaldehyde content of mackerel marketed in Dar es Salaam, Tanzania

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Abstract

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

Aim This study aimed to assess both the organoleptic quality and formaldehyde content of mackerels, the most consumed fish in Dar es Salaam, Tanzania.

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. Organoleptic quality was evaluated based on organoleptic characteristics. Formaldehyde analysis was done by High-Performance Liquid Chromatography (HPLC). Analysis of variance was subsequently run to test the variation of formaldehyde content in mackerel by outlet type and district.

Results All analyzed mackerel samples had acceptable levels of organoleptic quality (2.46 ± 0.50) and a mean formaldehyde concentration of 10.89 ± 2.44 mg/kg. On average, the samples from supermarkets had the highest level of organoleptic quality (2.20 ± 0.21) but were also 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 naturally formed formaldehyde in fish.

Conclusion Mackerels marketed in Dar es Salaam have acceptable organoleptic quality but are substantially contaminated with formaldehyde. Whether this is natural or artificial formaldehyde, our findings are inconclusive given the conflicting global standards. Nonetheless, the findings reveal the potential exposure of fish consumers to formaldehyde. Future research should explore the dynamics of the accumulation of natural formaldehyde in

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marketed fish and accurately assess the risk associated with the exposure of consumers to the formaldehyde in fish. The emanating findings will ultimately guide the development of local guidelines for natural and permissible formaldehyde concentrations in fish and fish products in Tanzania.

Introduction

Fish is a food with high nutritional value, being rich in protein, fat, minerals, and vitamins (Hoitsy et al. 2012; Mcmanus and Newton 2011). One major challenge with fish is its quick spoilage during storage periods (Mahmud et al. 2018). When exposed to high temperatures, moisture, and microbial contaminants, stored fish spoils and accumulates toxic substances in its flesh, including formaldehyde (Ghaly et al. 2010; Islam et al. 2015). In addition to this naturally occurring formaldehyde, unscrupulous traders are reported to deliberately add a 37–50% formaldehyde in water solution (known as formalin) to harvested fish and other perishable foods to increase shelf life (Islam et al. 2015; Paul et al. 2014; Saba et al. 2015).

This is risky to the consumers since formaldehyde is a group 1 carcinogen (IARC 2006; WHO 2001), and linked to nasopharyngeal cancer and myeloid leukemia (American Cancer Society 2014; Hauptmann et al. 2003; IARC 2006; Pinkerton et al. 2004; WHO 2001). In Tanzania, there are no scientific reports on the formaldehyde content of consumed fish, despite the growing speculations on its clandestine use in fish preservation by unfaithful traders. It is speculated that fish transported to distant markets or sold in supermarkets in Tanzania are treated with formalin as a preservative (Jamii forums 2022; Mwananchi Newspaper 2022).

The problem is also speculated in other African countries, although scientific reports originate from Ghana and Nigeria alone, where the formaldehyde contents of not more than 3.71 mg/kg of fish weight have been detected in both local and imported fish(Akipe et al. 2020; Asare-Donkor et al. 2018). Globally, most of the reports on formaldehyde detection in marketed fish originate in Asia. The reports, however, have debatable significance because of the conflicting country cutoff values for the naturally formed formaldehyde in fish (Das et al. 2018; Jaman et al. 2015; Jamila and Immaculate 2018; Joshi et al. 2015; Meida et al. 2020; Nowshad et al. 2018; Saba et al. 2015).

Nevertheless, formaldehyde, whether naturally formed or artificially added to fish, is hazardous when consumed above the established daily limit of 0.2 mg/kg body weight (Norliana et al. 2009). In Tanzania, there are concerns that chemicals, including formaldehyde, in perishable foods such as meat and fish (Jamii forums 2022), play a role in the growing cancer burden in the country (IARC 2019; MoHSW-Tanzania 2013).

Along the coastal strip of Tanzania, mackerel is the relatively cheaper, readily available, and most consumed fish. Like other fish in the Scombridae and Scomberesocidae families, mackerel is very perishable at tropical temperatures (Towers 2015). Considering the high temperatures along coastal Tanzania (18.1–32.4 ˚C) (WMO 2020), and the ongoing speculation that unscrupulous traders use formalin to preserve perishable food products, we investigated both the formaldehyde content and organoleptic quality of mackerel consumed in Dar es Salaam, the most populated coastal city in Tanzania.

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, namely Temeke, Ubungo, Kigamboni, Ilala, and Kinondoni (Fig. 1). 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.

Organoleptic quality evaluation

An adopted, subjective method that evaluates the organoleptic quality of fish was used (Howgate 2011; Patterson et al. 2014). The evaluated organoleptic characteristics included the odor of the neck, the odor and color of the gills, the general appearance of the fish, slime on the skin and eyes, as well as the texture of the fish. At least two blinded observers 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 organoleptic quality was obtained as the mean of the scores for each parameter. The organoleptic quality was established as excellent ($1<2$), acceptable/good ($2<5$), and unacceptable/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- 70,001 F, Japan) (HPLC) analyses were

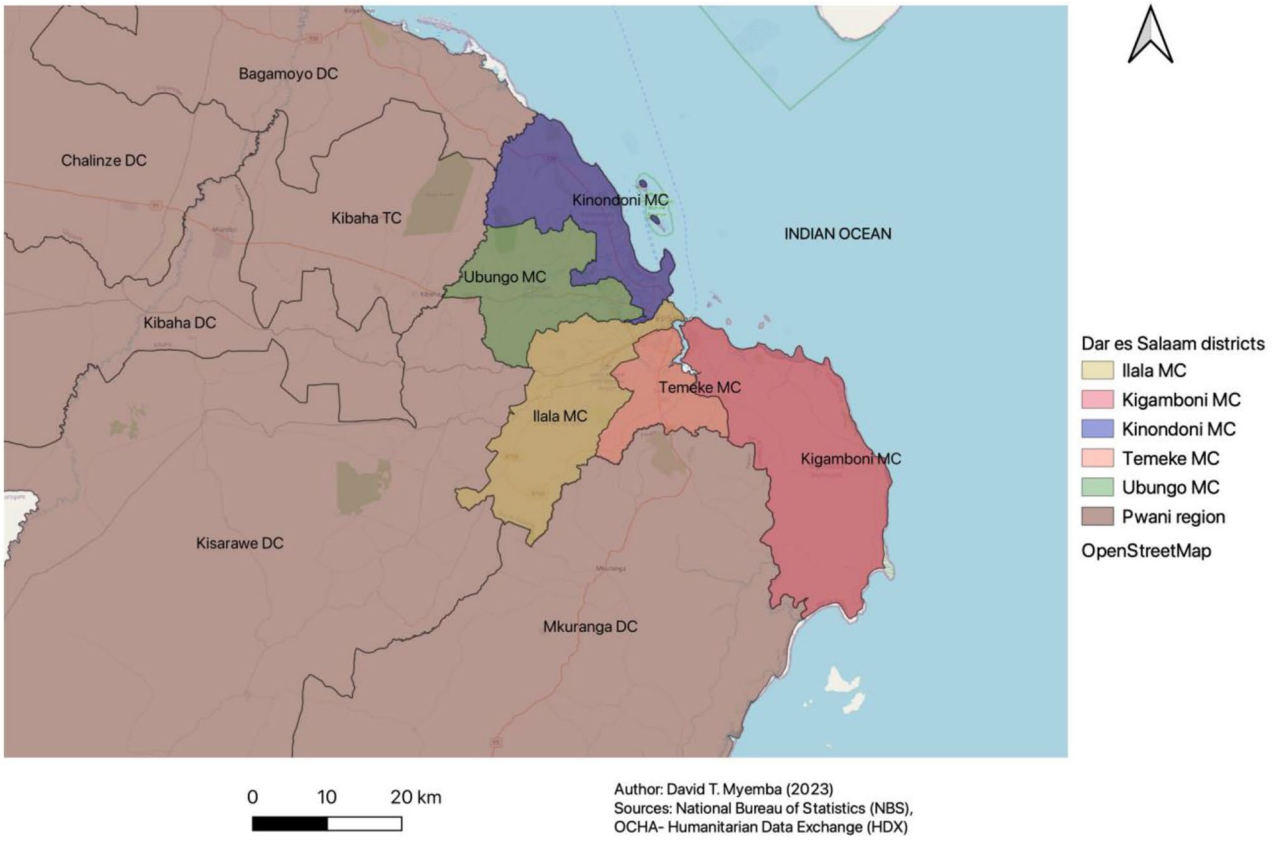

Fig. 1 Dar es Salaam map showing administrative districts

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 °C.

Preparation of the derivatizing agent

2,4-dinitrophenyl hydrazine (DNPH) 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 50 mL conical flask, followed by the addition of 5 mL of distilled water. The flask was then capped and sonicated for 40 min at 20 °C followed by centrifugation (HERMLE Labortechnik Z206A, Germany) at 7000 rpm for 10 min.

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 h at room temperature to allow the formation of orange hydrazone precipitate. 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, limit of detection, limit of quantification, as well as precision. Validation was performed according to the United States Food and Drug Administration protocol (FDA 2018). Table 1 and Fig. 2 show the method validation findings.

Table 1 Analytical Method validation findings

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

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). Moreover, the interaction of district and fish outlet (supermarket, local market and street vendors) factors on the concentration of formaldehyde was examined. The correlation of organoleptic quality of mackerel with the concentration of formaldehyde was analyzed as well.

Results

Organoleptic quality of mackerel

All mackerel samples exhibited acceptable levels of organoleptic quality (2.46 ± 0.50) (Table 2). Specifically, the samples obtained from supermarkets had the highest levels of organoleptic quality (2.20 ± 0.21) whereas those from street vendors had the lowest levels of organoleptic quality (2.83 ± 0.86). However, the observed differences were not statistically significant ($p = 0.055$).

Detection and quantification of formaldehyde in mackerel

The analyzed mackerel samples had mean formaldehyde 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 outlet types, the samples from supermarkets had the highest formaldehyde content (16.07 ± 4.68 mg/kg), whereas those from local markets had the least content (3.91 ± 1.86 mg/g). Based on source districts, fish samples from Ilala and Kigamboni, respectively, had the highest

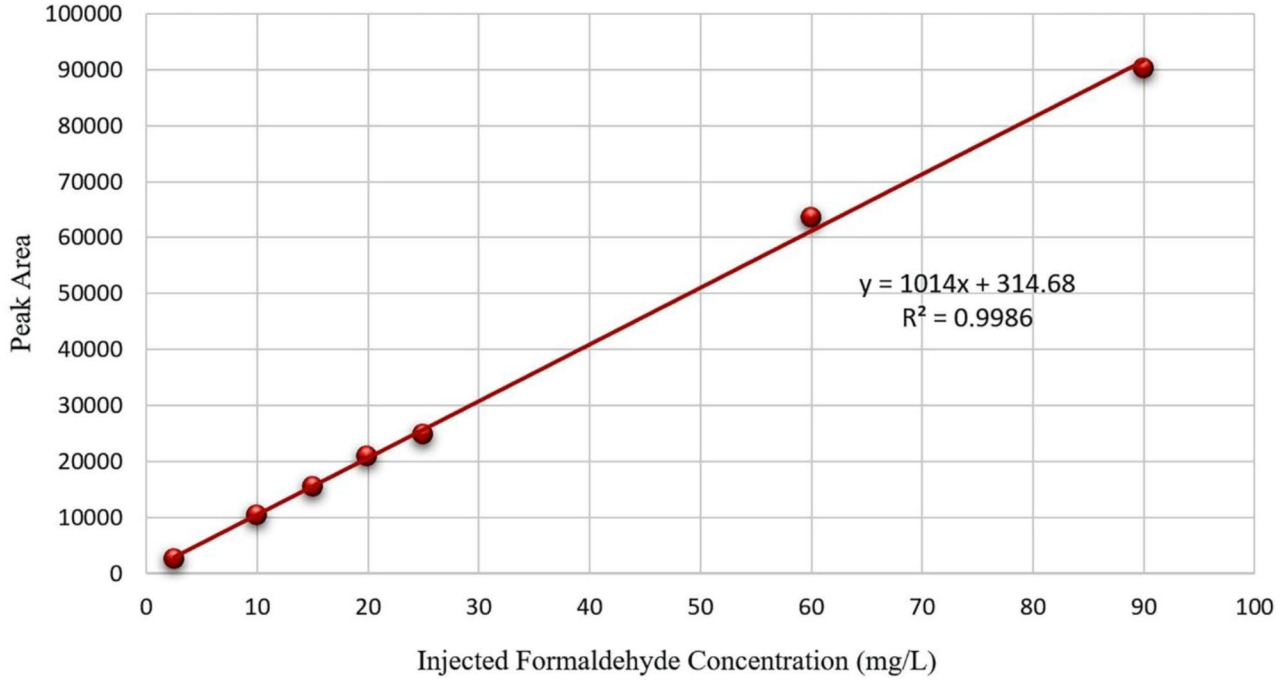

Fig. 2 Calibration curve for formaldehyde spiked on the control fish sample

Table 2 Organoleptic quality of mackerel samples from different fish outlets and Dar es Salaam districts

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**				

Table 3 Concentration of formaldehyde in mackerel 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 > 20 mg/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 > 20 mg/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**	

**** compared to the local markets means the difference is significant at p value < 0.0001, *** means the difference is significant at p value < 0.001

Table 4 Pearson correlation between mackerel organoleptic quality and formaldehyde content

		Formaldehyde concentration (mg/kg)
Organoleptic quality	Pearson Correlation	0.02
	Sig. (2-tailed)	0.96
	N	15

(13.84 ± 9.64 mg/kg) and least (7.13 ± 4.65 mg/kg) formaldehyde contents (Table 3).

Considering the previous estimation for the highest concentration of formaldehyde in fish flesh (20 mg/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). Moreover, the district and vendor factors did not have a statistically significant interaction on the formaldehyde content (p = 0.32).

Correlation analysis between organoleptic quality and formaldehyde

Upon correlation analysis, the organoleptic quality and the formaldehyde content of mackerel from all sources did not exhibit any correlation (r = 0.02) (Table 4).

Discussion

Organoleptic quality of mackerel consumed in Dar es Salaam

This study reports acceptable organoleptic quality of mackerel available in the Dar es Salaam markets. Despite the lack of statistical justification, we report a high level of organoleptic quality for fish obtained from supermarkets compared to those obtained from local markets and street vendors. This can be attributed to the availability of good freezing facilities (Botelho et al. 2013; Mylona et al. 2017; Wei and Sammalisto 2011) and probably the speculated misconduct preservation means in supermarkets (Jamii forums 2022; Mwananchi Newspaper 2022; The Citizen 2019).

Compared to those from the supermarkets, samples obtained from the local markets had a lower but acceptable organoleptic quality. That can be linked to the shorter storage periods of fish in those markets caused by high turnover rates. It is from those markets that the Dar es Salaam majority and low-income citizens obtain their food products at relatively affordable prices, which assures quick turnover. 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 organoleptic quality relatively acceptable (*personal observation*).

On the other side, samples obtained from the street vendors had the lowest level of organoleptic quality, and this can relate 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 organoleptic quality and accumulation of biochemicals in the fish muscles (Towers 2015).

Formaldehyde content of mackerel consumed in Dar es Salaam

Our findings reveal substantial formaldehyde content (10.89 ± 2.44 mg/kg) of mackerel consumed in Dar es Salaam, with 18.3% (11/60) of the samples having formaldehyde concentrations above the previously estimated upper limit of 20 mg/kg for the naturally formed formaldehyde in fish flesh (IARC 2006). The findings further reveal that the samples from supermarkets had

the highest formaldehyde content (16.07±4.68 mg/kg), whereby up to 35% of them had formaldehyde content above the estimated upper limit. On the other side, the lowest formaldehyde content (3.91±1.86 mg/kg) was associated with samples from the local markets, and none of them (0%) had formaldehyde content above the estimated limit.

The observed difference in formaldehyde content can partly be attributed to the high turnover rates in the local markets compared to the prolonged storage of fish in supermarkets, as described earlier. This limits the accumulation of natural formaldehyde in the fish marketed at local markets while allowing the accumulation of the same chemical in fish stored for long periods in supermarkets. Although our study cannot ascertain the origin of the detected formaldehyde, the high formaldehyde content of supermarket-obtained samples can partly be related to the speculated deliberate addition of formaldehyde, among other harmful chemicals, as a preservative to the stored fish (Jamii forums 2022; Mwananchi Newspaper 2022; The Citizen 2019; Wako 2019).

Notably, the fish formaldehyde contents observed in our study are relatively higher than those reported in Ghana and Malaysia, whereby formaldehyde has been detected in several fish species in the ranges of 0.174 to 3.71 mg/kg and 0.38 to 15.75 mg/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).

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 (Fig. 1). Such proximity minimizes the mentioned time interval above, and this can be a cause of the averagely lowest formaldehyde content (7.13±4.65 mg/kg) observed in fish sourced from the district as compared to the formaldehyde contents of fish from the other districts which were above 10 mg/kg. This therefore partly reflects the importance of quickly transferring and properly storing fish post-harvest in order to minimize the accumulation of natural formaldehyde.

Conclusion

Mackerels consumed in Dar es Salaam are of acceptable organoleptic quality but contain substantial amounts of formaldehyde. Based on the methodological approach used in this study and the conflicting global standards for natural formaldehyde in fish, we cannot ascertain whether the detected formaldehyde in the analyzed samples had accumulated naturally or was artificially added by the traders. Despite this uncertainty, these findings reveal the potential exposure of consumers to the formaldehyde in the consumed fish. Therefore, well-controlled studies should be conducted to study the natural accumulation of formaldehyde in marketed fish as a function of time, temperature, and humidity, among other storage conditions. In parallel to that, risk assessment studies should also be conducted to characterize both the actual consumer exposure to formaldehyde in fish and the burden of health problems attributable to this exposure. Ultimately, the generated data will inform the development of guidelines for natural and permissible levels of formaldehyde in fish and fish products marketed in Tanzania.

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

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

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Author contributions

All authors contributed significantly to the development of this manuscript. VPM 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.

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Data availability

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

Declarations

Ethical approval

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

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.

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