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Determination of formaldehyde in fresh seafood from Cambodia's local market

Report

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Abstract: Seafood is a rich nutritional food. It provides a healthy source of protein, minerals, and vitamins. To extend shelf life, formalin was added to seafood. Formaldehyde, or formalin, is an organic compound and has been widely used as a preservation food. The concentration of formaldehyde was considered according to food safety and human health. The aim of this study was the identification and determination of formaldehyde in fresh seafood from Cambodia's local market. The three samples of seafood—shrimp, squid, and short mackerel were analyzed using the UV spectrophotometry technique where the absorbance of formaldehyde was detected at 412 nm. Therefore, squid contains formaldehyde in the range of 3.199-0.164 mg/kg, shrimp 0.174-0.134 mg/kg, and short mackerel 0.168-0.140 mg/kg, following the findings of the formaldehyde investigation. The accuracy was presented as a recovery percentage. It exceeded 95%; that was between 80-120%, which represents good accuracy. In conclusion, the presence of formaldehyde in seafood and other foods should be properly investigated and monitored to detect this harmful chemical.

1. Introduction

Formaldehyde is a simple chemical compound that is made of carbon, hydrogen, and oxygen. This compound is a colorless, odorless, and irritating substance, and it is typically found as a 37–56% aqueous solution. Another crucial bioactive substance that is involved in many biological processes is formaldehyde. Two methods by which plant species can create formaldehyde are methyl transfer and demethylation, among other reactions (Klose et al., 2007). As part of their chemical communication, plants react to environmental stress by producing formaldehyde. Besides, formaldehyde is a secondary metabolite that is created during the synthesis of some chemicals and may have preventive or defensive properties. Plants may release formaldehyde as part of their stress response system in reaction to external stresses, including pathogen infections, climatic changes, or mechanical damage (Blande et al., 2014).

Formaldehyde, typically referred to as "formalin," is an inexpensive chemical that serves in multiple sectors of agriculture as a preservative and disinfectant. According to Kim et al., (2011), formaldehyde (FA) is a crucial and valuable chemical for the global economy, utilized across various industries such as construction (wood processing, furniture, textiles, carpeting) and consumer goods (antiseptics, medications, cosmetics, dishwashing liquids, glues, lacquer, etc.). One of the main forms of adulteration in many types of food is the unauthorized addition of FA to extend their shelf life (Jinadasa et al., 2022). Examples of these food groups include fish and shellfish, fruits and vegetables, fruit juice, mushrooms,

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and milk (Kamonchanok et al., 2022). Fishermen and vendors in Asian and European countries often add formaldehyde to fish to prolong shelf life and maintain freshness. Formalin's antimicrobial properties allow it to bind to shellfish proteins and produce muscular stiffness. One million cases of foodborne illness have been connected to contaminated and harmful foods (Kamonchanok et al., 2022). Human blood contains varying concentrations of formaldehyde (10 μM to 100 μM), with larger accumulations linked to organ aging (Wang & Zhang, 2023). High formaldehyde concentrations can cause cells to undergo more apoptosis or less mitosis (Wang & Zhang, 2023).

The presence of formalin in seafood concerns the food safety and health of the public. Fish, shrimp, and squid have been shown to be contaminated with formalin in several countries. In most cases, it results in severe stomach pain, vomiting, coma, nephritic damage, and even death. According to the World Health Organization (WHO), the major way that the general public is exposed to formaldehyde is by inhalation (WHO, 2010) The International Agency for Research on Cancer (IARC) has categorized formaldehyde as being human carcinogenic in Group 1. The World Health Organization, however, thought that the facts did not support the theory that aldehyde was a malignant neoplastic disease that was caused by biology. Crustacea will eventually perish since some marine fish will produce more aldehyde while being frozen. It was rumored that after being stored in cold storage, the levels in Bombay duck could have reached 400 mg/kg. Forms resulting from the ordinary metabolism of plants or animals have been shown to have different formaldehyde concentrations. Among them are fruits, vegetables, meat, fish, and other things. Moreover, fish flesh ages and breaks down because of a system that produces formaldehyde. The maximum daily dose reference (RfD) is 0.2 mg/kg body weight, which is stated by the United States Environmental Protection Agency (USEPA) (Laly, Priya, Panda, & Zynudheen, 2018).

However, in Cambodia, formaldehyde was banned from use in food products (Ministry of Commerce, 2006). Since seafood is a common food nowadays, people like to eat it raw and fresh. To implement preventive actions for safety and public health, FA detection is receiving an increasing amount of attention (Yang et al., 2024). Several methods for detecting formalin include high-performance liquid chromatography, electrochemical sensors, mass spectrometry, gas chromatography-mass spectrometry, chemiluminescence, and spectroscopy. Spectrophotometric TAC tests provide many advantages, such as inexpensive sample costs, rapid turnaround times, and the option to be performed manually, semi-automatically, or automatically (Kumar et al., 2022). This study aims to determine the concentration of formaldehyde in fresh seafood including short mackerel, shrimp, and squid from a local market located in Phnom Penh, Cambodia.

2. Materials and method

2.1. Chemicals

All the chemicals used were analytical grade. 6.0% (w/v) Trichloroacetic acid (TCA) was prepared by weighing 30.0 g and dissolved with 500.0 mL of distilled water. Nash's Reagent was prepared by mixing 0.2 mL of acetylacetone, acetic acid glacial, and ammonium acetate 15.0 g was dissolved in distilled water and adjusted to 100.0 mL. The reagent was stored in the dark at 0 °C due to its light sensitivity. 0.1 M NaOH was used to adjust the filtrate to around pH 7.0.

2.2. Standard curve

Formaldehyde stock solution (100.0 mg/kg) was diluted to 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 mg/kg in distilled water. Afterward, 5.0 mL of each standard solution was taken, along with 2.0 mL of Nash's

reagent. Each standard solution was put in the water bath at 60°C for 30 minutes. The absorbance was measured at 412 nm with a UV spectrophotometer (GENESYS 10S UV-VIS Model Dual-Beam Optics, Thermo Scientific, United States) to make a standard curve. Six formaldehyde concentrations were measured in duplicate.

2.3. Samples collection

Three seafoods, namely shrimp (*Penaeus monodon*), squid (*Uroteuthis chinensis*), and short mackerel (*Rastrelliger brachysoma*), were collected from Tror Loak Bek Market in Phnom Penh City, Cambodia. All samples were randomly selected from three different vendors, kept in a polyethylene bag, and immediately transported to the laboratory. Samples were analyzed as fresh without rinsing.

2.4. Formaldehyde determination

Shrimp, squid, and short mackerel were cut into small pieces (<5mm). 30.0 g of each sample was homogenized with 60.0 mL of 6.0% (w/v) TCA. The mixture was then filtered through a No. 1 filter paper (Whatman, England). A 5.0 mL of NaOH was added to the filtrate to adjust the pH to 7.0, and it was then incubated on ice for 30 minutes. 5.0 mL of the filtrate and 2.0 mL of Nash's reagent. The mixture was incubated again in the water bath at 60°C for 30 minutes. The absorbance of the samples was measured at 412 nm immediately by a UV spectrophotometer (GENESYS 10S UV-VIS Model Dual-Beam Optics, Thermo Scientific, United States) for formaldehyde determination. All experiments were done in triplicate.

2.5. Statistical analysis

All experiments were done in triplicate. The data are reported as mean ± standard deviation (SD), and the formaldehyde concentrations in samples were compared using one-way ANOVA, with statistical significance assigned if $p < 0.05$.

3. Result and discussion

3.1. Standard curve

The standard curve was prepared using the working standard solution of formaldehyde concentrations ranging from 0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 mg/kg. The relation between formaldehyde concentration and absorbance was linear with $R^2 = 0.9986$, and an equation of the standard curve is $y = 0.1805x + 0.0057$, where y is in absorbance units (AU) and x is formaldehyde concentration in mg/kg Figure 1. The limit of detection (LOD) and limit of quantification (LOQ) were calculated from the standard deviation of the lowest concentration and the slope of the calibration curve (European Commission, 2002). The formulas used for LOD and LOQ calculations were mentioned in Equations 1 and 2. The LOD of 0.017 mg/kg and LOQ of 0.055 mg/kg were achieved.

$$LOD = [(3 \times s)/m] \quad (1)$$

$$LOQ = [(10 \times s)/m] \quad (2)$$

where 'm' is the slope obtained from the standard curve, and 's' is the standard deviation of the lowest concentration, which was run 10 times.

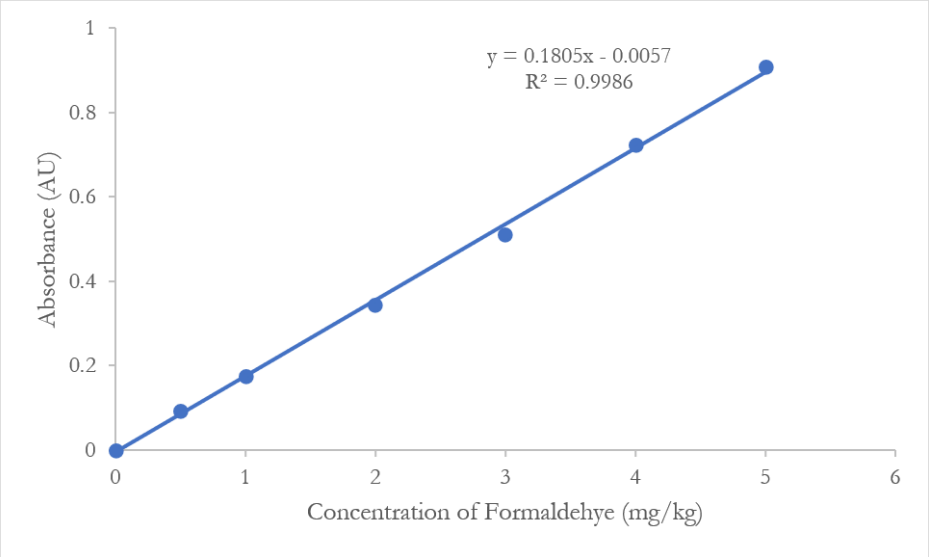

Figure 1. The standard curve of formaldehyde from 0.5 to 5.0 mg/kg measured at 412 nm

3.2. Determination of formaldehyde in samples

The Mean & SD values of formaldehyde in fresh samples of seafood such as shrimp, squid, and short mackerel are summarized in Table 1. The squid's formaldehyde content differs substantially between the retailers. In contrast to the amounts of 0.164 ± 0.006 mg/kg and 0.259 ± 0.048 mg/kg reported by Shops 1 and 2, Shop 3 had an unusually high concentration of 3.199 ± 0.023 mg/kg. Samples of shrimp from all three shops have a formaldehyde content, and the highest concentration is from Shop 2, which is 0.277 ± 0.030 mg/kg. The formaldehyde content of Shops 1 and 3 was found to be inconsistent, with reported quantities of 0.174 ± 0.064 mg/kg and 0.134 ± 0.012 mg/kg. Samples of short mackerel from all three shops have a formaldehyde content, while Shop 3 has a slightly higher concentration of formaldehyde, which is 0.168 ± 0.008 mg/kg.

Table 1. Formaldehyde concentration in seafood by number of samples without rinsing method used.

Seafood type	Formaldehyde concentration (mg/kg)					
	Sample (n = 9)					
	Shop 1	SD	Shop 2	SD	Shop 3	SD
Squid	0.164[a]	0.006	0.259[a]	0.048	3.199[a]	0.023
Shrimp	0.174[a]	0.064	0.277[a]	0.030	0.134[a]	0.012
Short mackerel	0.140[a]	0.017	0.155[a]	0.013	0.168[a]	0.008

Notes: Data are presented as the mean values; SD means standard deviation, Values with similar letters refer to "not significantly different" ($p > 0.05$; one way ANOVA).

The P-value of the samples indicates that there is no statistically significant difference in the formaldehyde content at the 95% confidence level. This lack of significance is consistent with the previously mentioned one-way ANOVA result, which showed no significant difference ($p > 0.05$) in formaldehyde concentrations among shrimp, squid, and short mackerel. Formaldehyde is not allowed to

be used in food products, but the fact that it shows up in every test raises serious concerns about public health. The results show that to guarantee consumer safety, strict oversight and control over seafood preservation methods are required. The sources of formaldehyde in seafood and the effects of various preservation techniques could be the subject of more investigation.

3.3 The accuracy of formaldehyde detection

The accuracy of the method was estimated by using the spiked method. The standard solution of formaldehyde at 0.1 mg/kg was added to shrimp samples. The accuracy was presented as percentage recovery (%Recovery), and it was calculated to determine the efficiency of the formaldehyde detection method. Table 2. shows that the calculated mean formaldehyde content for the unspiked and spiked samples is 0.189 ± 0.004 mg/kg and 0.303 ± 0.008 mg/kg, respectively. The recovery percentages exceeded 95%, which was between 80 - 120%, and demonstrated the good accuracy of the method. The percentage recovery is given by Equation 3.

$$\% \text{ Recovery} = \frac{C \text{ spiked sample} - C \text{ unspiked sample}}{C \text{ added}} \times 100 \quad (3)$$

where: C_{spiked} is the concentration found in the sample after fortification

$C_{unspiked}$ is the concentration found in the sample before fortification

C_{added} is the concentration used for fortification.

Table 2. % Recovery of formaldehyde in spiked seafood samples

Shrimp	Added (mg/kg)	Concentration (mg/kg)	Mean	SD	% Recovery
Unspiked	0	0.192	0.189	0.004	113.511
	0	0.187			
Spiked	0.1	0.297	0.303	0.008	
	0.1	0.308			

3.4 Recommendations and suggestions on formaldehyde usage

Strong enforcement and tracking methods want to be installed with the resources of regulatory bodies to deal with this problem. It takes commonplace inspections, laboratory sorting, and random sampling to find out and discourage the illegal use of formaldehyde (EPA, 2012). Educating the general public in the area of the risks of formaldehyde in food products is critical (NIOSH, 2021). It is vital to tell providers and customers about the dangers this chemical poses to health and the importance of food safety legal hints. Campaigns that educate humans on how to apprehend safe food products and pressure the cost of purchasing from professional assets can enhance patron training (Consumer Reports, 2015).

For food suppliers, formaldehyde is a chemical that is used for germicide and prevention of their food for sale, but using this chemical in food products has become a serious accusation because of potential fitness risks. Formaldehyde has hazardous effects, which can have dire effects on human health, including respiratory problems, pore and skin infections, and cancer (IARC, 2006). Formaldehyde is a clear, can burn, strong-smelling stuff used in building things and making stuff for the house. It's in things like pressed wood, glue, fabric stuff, paper stuff, and some insulation. Also, it's used to fight stuff like germs

and mold in industry and as a preserver in places like mortuaries. It's also made in small bits by living things as part of how they live (IARC, 2006). Food aids cannot include formaldehyde in several world locations, including Cambodia. The Cambodian Ministry of Commerce banned the use of formaldehyde in food products with effect from September 28, 2006 (Ministry of Commerce, 2006). This preference eventually becomes part of a wider technique for ensuring the protection of food and the general health of the public. Rules say no formaldehyde in food. Any amount of this bad stuff is not okay, but stopping it is hard. Some bad people in the food industry might use formaldehyde to make things look better or last longer (WHO, 2010).

To make sure all the sellers and the public know how to keep food safe, we might need to offer classes such as workshops, seminars, or TV advertisements that relate to food contamination. These shows can deal with some topics, which include the importance of ordinary testing for infection and the secure handling and storage of food (FDA, 2019). To make certain that buyers are privy to the policies and recommended practices for food protection, it is required to provide schooling publications. These indicate that they can deal with several topics, which encompass the importance of sorting out meals every day for contamination and the safe management and storage of meals.

4. Conclusion

The potential health effects of formaldehyde or formalin, a carcinogen, on consumers are a concern when it is found in seafood (WHO, 2010). According to the method across the UV-spectrophotometer wavelength range, the results showed that the concentration of formaldehyde contained in squid had the highest amount of formalin among those two samples, shrimp and mackerel. The formaldehyde concentration of the squid varies greatly throughout the marketplace. In addition to Shops 1 and 2, which reported concentrations of 0.164 ± 0.006 mg/kg and 0.259 ± 0.048 mg/kg, Shop 3 had an exceptionally high concentration of 3.199 ± 0.023 mg/kg. The accuracy was presented as a recovery percentage. It ranges between 80-120%, which is above 95% and shows good accuracy. Consumers should be aware of the potential risks associated with seafood consumption and take the necessary precautions. To learn more about it and create practical strategies for its elimination or reduction, more research is required, and this should contribute to the development of safer and healthier food preservation techniques (FAO, 2009; EFSA, 2014).

Author contributions

Conceptualization, Chhun.S, Soknea.S.; methodology, Chao.S, Ann.S.; software, Chao.B.; validation, Chao. B, Soknea.S.; formal analysis, Soknea.S, Chao. B, Makara.P.; investigation, Soknea.S, Chao. B, Makara.P.; resources, Chhun, S.; data curation, Chhun.S, Chao.B.; writing—original draft preparation, Soknea.S, Makara. P, Chao.B.; Writing—review and editing, Soknea.S, Makara. P, Chao. B, Chhun.S.; visualization, Soknea.S, Makara. P, Chao. B, Chhun.S.; supervision, Chhun.S.

Data availability

Not applicable

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Conflicts of interest

Authors declare no conflicts of interest.

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