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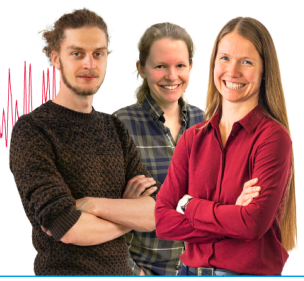

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The Investigation and Isolation of Histamine in Fish Performing Microwave-Assisted Extraction (MAE) and Analyzed Using Liquid and Gas Chromatography

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Abstract: Histamine is a compound that is related to food poisoning. The FDA has warned that histamine compound is acceptable below 50 $mg \cdot kg^{-1}$. For this reason, a swift, robust, and precise approach is required. To reach the criteria, chromatography methods have been applied, such as liquid chromatography (LC) equipped with fluorescence detector (FLD) and gas chromatography (GC) with mass spectrometry (MS). Histamine in fish samples was satisfactorily isolated with trichloroacetic acid (TCA) using microwave-assisted extraction (MAE). In terms of validation, the excellent linear range obtained between $10^{-3} – 10^{-1}$ $nmol \cdot L^{-1}$, satisfactory recovery at level 0.1 $nmol \cdot L^{-1}$ attained with detection limit at 0.01 $nmol \cdot mL^{-1}$. The validated methods were successfully applied to analyze histamine in fish samples. The MS detector was also successfully used to determine the molecular weight of histamine in fish tuna (*Thunnus sp*.).

Keywords: Histamine, isolation, chromatography, validation

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INTRODUCTION

Food is a fundamental human requirement, so food availability needs serious attention in quantity and quality. Food must be a concern because this matter is the most basic need, besides clothing and boards. The government's attention to food availability is carried out through a food security program so that people have sufficient, safe, nutritious, healthy, and halal food. If needs are met, quantity and quality at the individual and household level will ensure a healthy, active, and sustainable quality of life. They can avoid health and nutrition problems (Dasgupta & Robinson 2022; Geng et al. 2022).

Food detection becomes a considerable challenge, particularly for food industry sectors, because they act as producers and distributors. The food industries have a significant role in food distribution. Thus, they must ensure the food is secure before the consumers purchase and consume it. Numerous indicators require food to be secured and detected, such as food mishandling, the appearance of bacteria in foods, and the decrease in food quality owing to various indicators such as storage, temperature, and pH. Food proteins are built by several amino acids, and the amino acids can convert to a specific compound known as biogenic amines. It can be concluded that food proteins have biogenic amine compounds (Munir et al., 2023; Inayatullah et al., 2021).

The decarboxylation of amino acids is responsible for biogenic amine formations, and the biogenic amines are divided into three different structures: aliphatic, aromatic, and heterocyclic. The removal of the carboxyl group from amino acids causes several biogenic amine formations such as lysine to cadaverine, tyrosine to tyramine, tryptophan to tryptamine, histidine to histamine (Tiris et al. 2023; Lappa et al. 2022).

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Many academicians agree that histamine is one of the biogenic amine compounds generally traced in the human body, even at trim levels. Nevertheless, the Food and Drug Administration stated that histamine is consumed regularly and accumulated until 50 $mg \cdot kg^{-1}$, causing several adverse effects. However, our body has a security system that can detoxify histamine using histamine-N-methyltransferase (HMT) and diamine oxidase (DAO). At the same time, the presence of putrescine and cadaverine is able to modify the histamine from inactive to actively toxic (Boulfekhar et al., 2023; Moniente et al., 2022). There is a study about biogenic amine degradation where the biogenic amines can be degraded using the heating step, yet only histamine can survive in high temperatures (Clabretta et al. 2022).

Considering the abovementioned issue, many sectors, such as institutions, academicians, and food industries, should be involved in handling, studying, and monitoring food security. They must obtain the best approach to determine biogenic amines in foods, particularly histamine. This detection is imperative due to several factors, such as modifying the obsolete technique, reporting the foods commonly containing histamine, and so on (Bogdanovic et al. 2020). The histamine may be related to scombroid poisoning due to eating fish from the *Scombroidae* family, such as sardines, mackerel, tuna (*Thunnus sp*.), and mahi-mahi. Nevertheless, many studies have reported that many foods and proteins can produce histamine besides the *Scombroidae* family (Qiao et al. 2020).

Numerous techniques have been performed to investigate histamine levels in various samples, such as thin-layer chromatography (TLC), electrochemical sensor, spectrophotometry UV, colorimetry, electrophoresis, liquid and gas chromatography (LC & GC) (Koo & Lim 2023; Kowtharapu et al. 2022). Chromatography approaches such as LC and GC become the famous method for histamine detection. Therefore, the LC equipped with a fluorescence detector (FLD) was applied in this study, whereas the GC/MS was used for confirmation structure. The histamine detection was applied without a derivatizing process. The employed technique was validated regarding selectivity, precision, detection, and quantification limit, while the accuracy was validated using the fish tuna (*Thunnus sp*.).

MATERIALS AND METHODS

Standard chemicals and reagents

Histamine dihydrochloride (HIS; 99%) obtained from Sigma-Aldrich Indonesia. Trichloroacetic acid (TCA, 99%), water, and acetonitrile were HPLC grade purchased from Merck, Indonesia. As a solvent, the histamine stock solution was provided at 29.51 $nmol \cdot L^{-1}$ in HPLC-grade water. All chemicals and reagents employed are analytical and chromatographic grade purchased from Sigma Aldrich, respectively.

Preparation of standard solution and series concentrations of histamine

Stock solution (29.51 $nmol \cdot L^{-1}$) of histamine was prepared in HPLC grade water in a volumetric flask (10 mL), and stored in the icebox at 4ºC. Series concentrations of histamine were prepared from histamine stock solution and employed to establish the calibration curves (10^{-3} – 10^{-1} $nmol \cdot L^{-1}$).

Sample preparation

The extraction of histamine in tuna (*Thunnus sp.*) using the microwave-assisted extraction (MAE) technique. 5 g of tuna (*Thunnus sp.*) was scaled and homogenized in a beaker glass with 10 ml of TCA with different concentrations, such as 0.1% and 0.5%. The microwave-assisted extraction (MAE) was performed in this study to isolate the histamine compound in the tuna (*Thunnus sp.*) using several parameters such as the constant microwave frequency, 2450 MHz, the maximum power, 700 W, and the maximum time, 30 min. Approximately 5 g of the sample weighed. Afterwards, the sample was handled using a particular treatment. Nevertheless, before the treatment, a TCA was taken as the solvent. After that, the treatment was continued when the first temperature applied was 20ºC. After the treatment was finished, the final temperature was recorded. Afterwards, the sample was centrifuged at 2500 rpm for 10 min at 5ºC. The supernatant was taken using a micro-pipette for further analysis. A specific diagram of the MAE approach of the histamine substance of tuna (*Thunnus sp.*) is displayed in Figure 1.

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