# Identification of Volatile Compounds in Moringa Pods (*Moringa oleifera*) During Cooking Using HS-SPME Coupled with GC-MS

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**Short Report**

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# Abstract

Moringa (*Moringa oleifera*) is renowned for its therapeutic properties, with the chemical constituents of various plant parts well-documented. However, no study has previously explored the volatile compounds responsible for the characteristic aroma released during cooking of moringa pods, which is commonly practiced in South Indian cuisine "*Sambhar.*"This study aimed to investigate these volatile compounds using headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography–mass spectrometry (GC-MS). Moringa fruits (variety PKM-1) were harvested during their peak season and prepared using a traditional cooking method to release the aroma volatiles. These volatile compounds were analysed from both the headspace and the boiled water extract. The results revealed the predominant presence of nizatidine (CAS No.: 76963-41-2), an H2-receptor antagonist commonly used to treat ulcers, in the volatile headspace and in the boiled water extract. This discovery suggests that moringa pods might offer gastrointestinal therapeutic benefits, potentially due to the bioavailability of Nizatidine when consumed as part of regular meals. This study contributes to a deeper understanding of the volatile compounds in moringa pods and proposes further investigation into their therapeutic potential.


# 1. Introduction

## 1.1 Background

*Moringa oleifera*, often dubbed the "miracle tree," is a highly valued plant for its medicinal, nutritional, and therapeutic properties (Pareek et al. 2023). Native to India and grown worldwide, moringa is a rich source of vitamins, minerals, and bioactive compounds, which have been the subject of extensive research. Various parts of the plant, including the leaves, seeds, bark, and roots, have been examined for their chemical composition, contributing to a wide range of applications in health, nutrition, and traditional medicine (Ayoade et al. 2019). However, despite the extensive characterization of moringa's bioactive compounds, little attention has been paid to the volatile compounds that are released during the cooking of moringa pods, particularly in traditional South Indian cuisine. The study of volatiles is crucial for understanding not only the sensory characteristics of moringa but also the possible health benefits of compounds released during cooking (Zhang et al. 2022; Scortichini et al. 2020).

## 1.2 Importance of Volatile Compounds in Foods

Volatile compounds are responsible for the characteristic aromas of food and play an essential role in flavour perception (Tournier et al. 2007). Volatile components can originate from various chemical pathways, such as lipid oxidation, Maillard reactions, and enzymatic processes (Shakoor et al. 2022). These compounds can also contribute to the nutritional and medicinal qualities of foods. Several other volatile compounds have been identified as possessing antimicrobial, antioxidant, or anti-inflammatory properties (El Hachlafi et al. 2023).

Headspace solid-phase microextraction (HS-SPME) coupled with gas chromatography-mass spectrometry (GC-MS) has become a powerful analytical tool for identifying volatile organic compounds (VOCs) in various foods and plants. This method offers a non-destructive approach to capturing and analyzing the aroma profile of foods during cooking or processing. Prior studies have examined VOCs in fruits, vegetables, and spices (Lasekan and Azeez 2014), but no research has explored the volatile compounds released from moringa pods during cooking.

# 1.3 Significance of the Study

In this study, we aimed to investigate the volatile profile of moringa pods (*Moringa oleifera*, variety PKM-1) using HS-SPME and GC-MS. The cooking process was designed to replicate the traditional preparation method for "Sambhar," a lentil-based South Indian dish, to ensure the natural release of volatiles. Our findings provide novel insights into the chemical composition of moringa pods, particularly the unexpected detection of nizatidine, a histamine H2-receptor antagonist used in anti-ulcer medication. Understanding the presence and effects of such bioactive compounds can contribute to moringa's growing reputation as a functional food with both nutritional and medicinal properties.

## 2. Literature Review

# 2.1 Therapeutic Properties of Moringa oleifera

Moringa oleifera has been widely researched for its pharmacological potential. Studies have shown that the plant contains numerous bioactive compounds, including flavonoids, alkaloids, saponins, tannins, and glucosinolates (Anwar et al. 2007). These compounds have been associated with various therapeutic activities such as antioxidant, anti-inflammatory, anticancer, and antimicrobial effects.

Among the most significant discoveries in moringa research is its potential as a natural remedy for chronic diseases such as diabetes, hypertension, and hyperlipidemia (Mbikay 2012). Moringa leaves have been reported to lower blood glucose levels and improve insulin sensitivity, suggesting their role in managing type 2 diabetes. The seeds and pods have also been shown to have hypocholesterolemic properties, aiding in the reduction of high cholesterol levels (Leone et al. 2016).

# 2.2 Volatile Compounds in Vegetables

The volatile components of vegetables are crucial not only for their aroma but also for their health benefits. Studies have shown that cooking can significantly alter the volatile profile of vegetables, affecting both flavor and nutrient composition (Gong et al. 2023). Sulfur-containing compounds in cruciferous vegetables, such as broccoli and cabbage, have been shown to have anticarcinogenic properties (Vermeulen et al. 2008). These compounds are often released during cooking and can contribute to the overall therapeutic benefits of the vegetables.

While the volatile compounds of many vegetables have been extensively studied, research on moringa's volatile profile is limited. Previous studies have primarily focused on the nutritional content and pharmacological effects of moringa, with little attention paid to the aroma compounds released during cooking. This study aims to fill this gap by analyzing the volatile compounds released during the cooking of moringa pods.

# 2.3 Headspace-Solid Phase Microextraction (HS-SPME) and Gas Chromatography-Mass Spectrometry (GC-MS) in Food Analysis

HS-SPME is a widely used technique for the extraction of volatile compounds in food matrices (Belliardo et al. 2006). This method allows for the non-destructive extraction of volatiles from the headspace of a sample, providing a snapshot of the aroma compounds present in a given food item. The use of HS-SPME coupled with GC-MS has become the standard for volatile compound analysis due to its sensitivity and ability to identify and quantify compounds present in trace amounts.

GC-MS is a powerful analytical tool that separates volatile compounds based on their chemical properties and identifies them using mass spectrometry. The combination of these techniques has been employed in numerous studies to analyze the volatile profiles of foods such as fruits, vegetables, meats, and dairy products (Reineccius 2010). This study employs HS-SPME and GC-MS to analyze the volatile compounds released during the cooking of moringa pods.

## 3. Materials and Methods

# 3.1 Sample Collection and Preparation

Moringa pods of the variety PKM-1 were harvested during the winter season (November-December) in Coimbatore, Tamil Nadu, South India. This period was selected based on prior studies indicating that the chemical constituents of moringa are at their peak during winter (Rastogi et al. 2024). The pods were washed thoroughly with running tap water to remove surface dust and other contaminants.

# 3.2 Preparation of Samples

To mimic traditional cooking practices, the moringa pods were prepared using a method common in South Indian cuisine for the dish "Sambhar." The pods were cut into 2-inch pieces. In a 250 mL conical flask, 50 mL of deionized distilled water was heated until boiling. Six pieces of moringa pods were added to the boiling water, and the flask was immediately sealed with an extraction fiber to trap the volatiles.

# 3.3 Headspace-Solid Phase Microextraction (HS-SPME)

Volatile compounds were collected using headspace solid-phase microextraction (HS-SPME). The extraction fiber was inserted into the headspace of the conical flask immediately after adding the moringa pods to the boiling water and allowed to equilibrate for 10 minutes while the water continued to boil. After 10 minutes of exposure, the fiber was then removed, and the adsorbed volatiles were analyzed using gas chromatography–mass spectrometry (GC-MS).

# 3.4 Gas Chromatography-Mass Spectrometry (GC-MS)

The Clarus SQ 8C Gas Chromatography - Mass Spectrometer from Perkin Elmer, was engaged for the analysis. The instrument was set as follows, Injector port temperature set to 220˚ C, Interface temperature set as 250˚ C, source kept at 220˚ C. The oven temperature programmed as available, 75˚ C for 2 mins, 150˚ C @ 10˚ C/min, up to 250˚ C @ 10˚ C/min. Split ratio set as 1:12 and the injector used was splitless mode. The DB-5 MS capillary standard non - polar column was used whose dimensions were 0.25mm OD x 0.25μm ID x 30 meters length procured from Agilent Co., USA. Helium was used as the carrier gas at 1 ml/min. The MS was set to scan from 50 to 550 Da. The source was maintained at 220˚ C and 4.5e-6 mtorr vacuum pressure. The ionization energy was – 70eV. The MS was also having inbuilt pre-filter which reduced the neutral particles. The data system has inbuilt libraries for searching and matching the spectrum. NIST MS Search 2.2v contain more than five lakh references.

Interpretation of mass spectrum of GC – MS was done using the database of National Institute Standard and Technology (NIST14). The spectrum of the known component was compared with the spectrum of the known components stored in the inbuilt library.

# 3.5 Analysis of Boiled Water Extract

In addition to the volatile headspace analysis, the boiled water extract was also analyzed using GC-MS system to compare the volatiles released during cooking with those present in the water after boiling. This provided a comprehensive profile of both volatile and water-soluble compounds in the moringa pods.

## 4. Results

# 4.1 Headspace Volatile Compounds

The GC-MS analysis of the headspace volatiles revealed a complex profile of compounds, with a predominant peak at a retention time of 2.194 minutes corresponding to nizatidine (CAS No.: 76963-41-2). The mass spectra of this peak matched the library entry for nizatidine, a known histamine H2-receptor antagonist commonly used in the treatment of ulcers, with a confidence level of 95%, confirming its presence in the volatile compounds emitted during the cooking of moringa pods.

# 4.2 Boiled Water Extract Volatile Compounds

The GC-MS analysis of the boiled water extract also showed the presence of nizatidine, with a retention time of 2.179 minutes. The identification of nizatidine in both the volatile and aqueous phases suggests that this compound is released during the cooking process and remains stable in both forms.

# 4.3 Comparison of Volatile Profiles

Table 1 presents a comparison of the volatile compounds detected in the headspace and boiled water extract. Nizatidine was the predominant compound in both samples, while other minor compounds such as aldehydes, ketones, and esters were detected in trace amounts.

Table 1
Volatile Compounds Identified in Headspace and Boiled Water Extract. (only compounds with peak area > 1% are listed)

| Compound | Retention Time (min) | Headspace (Peak Area) | Boiled Water (Peak Area) | Suggested use |
|---|---|---|---|---|
| Cyclohexanone, (2-nitrophenyl)hydrazone | 1.984 | Not detected | 1.495 | unknown |
| Nizatidine | 2.179 | 89.805 | 38.908 | Anti-ulcer |
| Carbon disulfide | 2.339 | 2.533 | Not detected | floatation agent |
| Trichloromethane | 2.634 | Not detected | 3.016 | solvent |
| Ethane, 1,1-diethoxy- | 3.439 | Not detected | 5.088 | flavoring component of distilled beverages |
| 1,3,8-p-Menthatriene | 8.396 | 1.744 | Not detected | volatile oil component |
| Phthalic acid, 5-methylhex-2-yl heptadecyl ester | 19.551 | Not detected | 1.214 | plasticizers |

# 4.4 Comparison with standard

Nizatidine, from the commercially available capsule Axid, was used as a standard for GC-MS elution time analysis. Boiling of Axid (10mg) in 100 ml distilled water gave a very faint aroma of boiling moringa pod. It is possible that other compounds in moringa pods might also act synergistically to give-out the characteristic aroma.

## 5. Discussion

# 5.1 Implications of Nizatidine Detection

The detection of Nizatidine in the volatile profile of moringa pods is a novel finding. This compound, known for its pharmacological action as an H2-receptor antagonist, is typically used to treat gastrointestinal conditions such as ulcers and GERD. Its presence in the volatile compounds of moringa pods suggests that these pods may offer additional therapeutic benefits, particularly for gastrointestinal health. Nizatidine's role as a bioactive compound in food has not been previously documented, making this discovery significant in the field of functional foods.

# 5.2 Potential Bioavailability of Nizatidine in Moringa Pods

The stability of nizatidine in both the volatile and aqueous phases indicates that this compound is not only released during cooking but may also contribute to the therapeutic properties of moringa when consumed. This finding is consistent with previous research on the bioactive compounds in moringa, which have demonstrated a wide range of medicinal benefits, including anti-inflammatory, antimicrobial, and anticancer properties (Su et al. 2023). However, the discovery of nizatidine adds a new dimension to the understanding of moringa's pharmacological potential.

## 5.3 Comparison with Other Vegetables

The presence of pharmacologically active volatile compounds in vegetables is not uncommon. For example, glucosinolates in cruciferous vegetables such as broccoli and cabbage are known for their anti-cancer properties (Vermeulen et al. 2008). The discovery of Nizatidine in moringa pods adds to the growing body of evidence that volatile compounds in vegetables can contribute to their medicinal value. However, the bioavailability of nizatidine from moringa pods when consumed as food should be investigated to assess its potential therapeutic benefits.

## 6. Conclusion

This study provides the first evidence of nizatidine in moringa pods during cooking, detected using HS-SPME coupled with GC-MS. The identification of nizatidine in both the volatile headspace and the boiled water extract highlights the potential medicinal applications of moringa, beyond its traditional nutritional uses. Given the pharmacological properties of nizatidine, moringa pods may offer a natural source of this compound, which could have implications for dietary supplementation and alternative medicine. Future studies should explore the broader occurrence of nizatidine in plant-based foods and its bioactive effects *in vivo*.

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# Figures

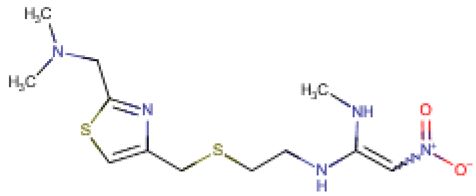


Figure 1

Chemical structure of Nizatidine.

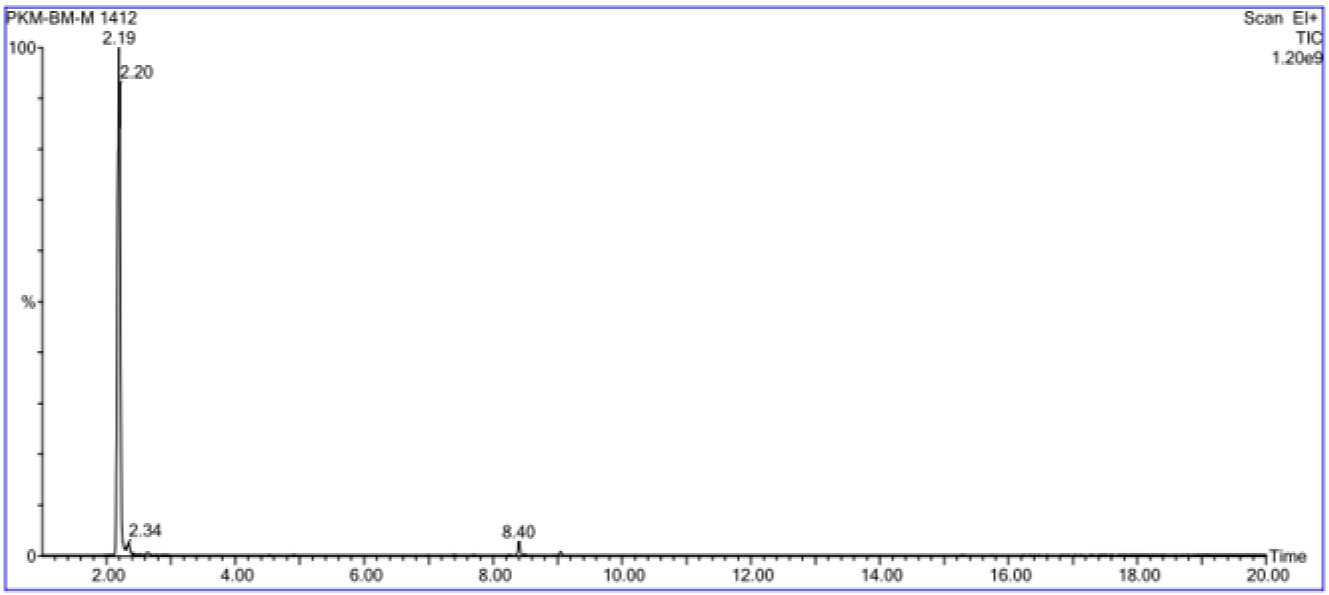


Figure 2

Chromatogram from the headspace GC-MS analysis.

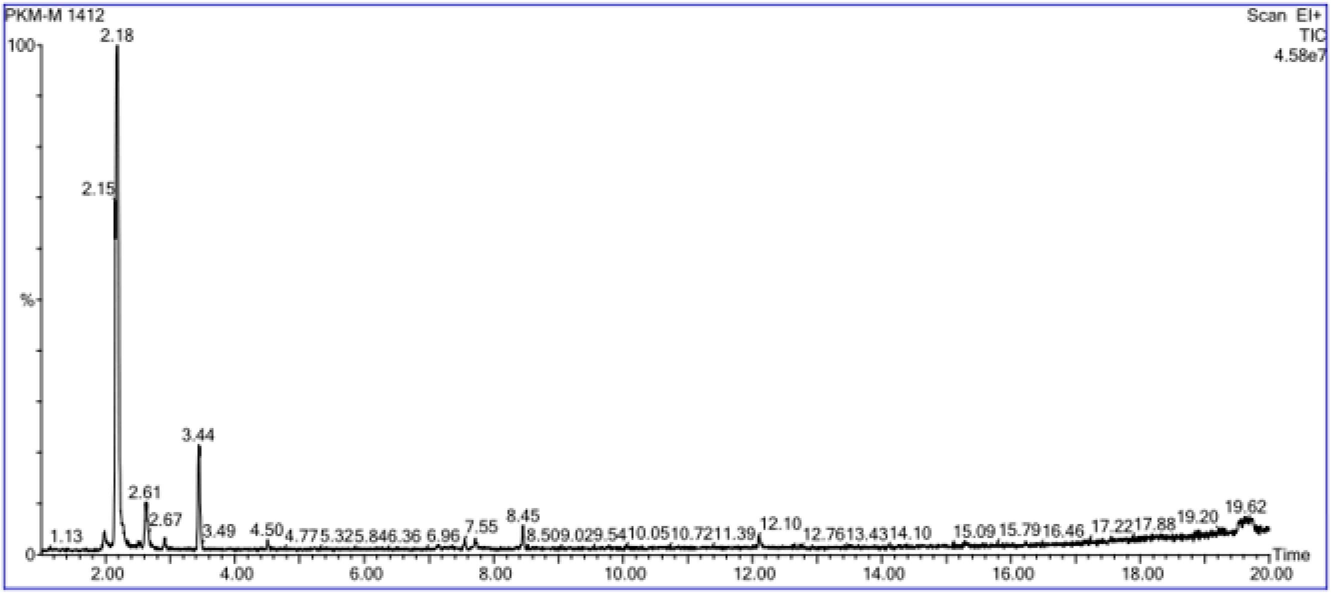


Figure 3

Chromatogram from the boiled water extract GC-MS analysis.