

Phytochemical Profiling of Bioactive Compounds in Ethanolic Extract of Black Garlic (*Allium sativum* L.) using GC-MS Analysis

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Abstract

Black garlic (*Allium sativum* L) is produced by aging fresh garlic under controlled temperature and humidity conditions resulting in significant biochemical transformations and enrichment of bioactive compounds. The aging process promotes the formation of stable metabolites through Maillard reaction and other thermal processes enhancing the antioxidant and therapeutic potential of garlic. Phytochemical profiling of black garlic is therefore essential to understand the compounds responsible for its biological activities

Keywords

Black garlic, phytochemical profiling, Maillard reaction, functional foods

Introduction

Garlic (*Allium sativum L*) has been widely recognized as a medicinal plant due to its numerous pharmacological properties including antioxidant, antimicrobial and anti-inflammatory and anti-cancer activities. These biological effects are largely attributed to the presence of organosulfur compounds, polyphenols, flavonoids and other bioactive phytochemicals present in garlic. In recent years increasing attention has been given to black garlic a processed form of fresh garlic produced through controlled thermal aging under high temperature and humidity conditions. During this aging process complex biochemical reactions occur particularly the Maillard reaction which leads to the transformation of unstable compounds such as allicin into more stable and biologically active molecules

Compared with fresh garlic black garlic (Figure 1) exhibits enhanced antioxidant capacity improved nutritional value and reduced pungency. These changes are mainly associated with the formation of new phytochemical compounds including phenolics, flavonoids, organic acids and Maillard reaction products. Several studies have demonstrated that thermal processing significantly modifies the chemical composition of garlic resulting in the generation of compounds that contributes to its improved biological activity.

Identification of phytochemical constituents in plant extracts plays an important role in understanding their pharmacological potential. GC-MS is one of the most widely used analytical techniques for the qualitative identification of volatile and semi-volatile compounds present in the plant extracts. GC-MS analysis enables accurate identification of bioactive compounds based on retention time and mass spectral comparison with reference libraries

Therefore, the present study aimed to identify the phytochemical constituents present in the ethanolic extract of black garlic using GC-MS analysis. Identification of these compounds may provide insights into the bioactive components responsible for the antioxidant and hepatoprotective properties associated with black garlic.

Methodology

Sample procurement and extraction

Black garlic samples were obtained from the local market and dried under laboratory conditions prior to extraction. Ten cloves of black garlic were selected and carefully peeled. The cloves were weighed before and after peeling to standardize the concentration of the sample. The peeled cloves were then soaked in 70:30 ethanol solution and incubated for 24 hrs at 40°C to facilitate extraction of phytochemical compounds.

After incubation the extract was filtered using Whatman No.1 filter paper to remove solid residues. The filtrate obtained was concentrated and dried under appropriate conditions. The dried extract was stored at controlled temperature until further analysis.

GC-MS analysis

The phytochemical composition of the ethanolic extract of black garlic was analyzed using GC-MS. The analysis was carried out using a Thermo Scientific Trace GC system coupled with a DSQ mass spectrometer operating under electron impact ionization at 70 eV.

Separation of compounds was achieved using a DB-5MS column (30m length, 0.25 mm internal diameter and 0.25 µm film thickness). The oven temperature was initially maintained at 50°C for 1 min followed by a temperature increase at a rate of 10°C per minute upto 280°C where it was held for 15 minutes. The injector and detector temperatures were maintained at 250°C and 280°C respectively. Helium (99%) was used as the carrier gas at a constant flow rate of 1 mL/min. The mass spectra were recorded in the range of m/z 45-500 with a scan rate of 5.1 scans per second. Identification of compounds was carried out by comparing their mass spectra and retention time with those available in standard spectral libraries.

Results and discussion

The GC-MS analysis of the ethanolic extract of black garlic revealed the presence of several phytochemical compounds belonging to different chemical classes such as fatty acids, aldehydes, esters, heterocyclic compounds and carbohydrate derivatives. The identified compounds were characterized based on their retention time, molecular formula and molecular weight through comparison with reference spectral databases.

A total of 19 compounds (Table 1) were detected in the ethanolic extract including the presence of a diverse range of bioactive molecules formed during the aging process of garlic. The identified compounds include ethanol, 5-methyloxazolidine, furfural, 3-furaldehyde, 6-acetyl- β -D-mannose, 2,5 dimethyl-4-hydroxy-3(2H)-furanone, 3-furancarboxylic acid methyl ester, 4H-pyran-4-one derivatives, diethyl phthalate, n-hexadecanoic acid, oleic acid and several fatty acid methyl esters. Among these compounds 6-acetyl- β -D-mannose was identified as one of the major carbohydrate derived compounds. Such compounds are commonly formed during the Maillard reaction between amino acids and reducing sugars during the thermal processing of garlic. The presence of these compounds contributes to the characteristic color, flavor and antioxidant activity of black garlic.

Fatty acids and their derivatives were also prominently detected in the extract. These include n-hexadecanoic acid (palmitic acid) and oleic acid which are well known for their biological activities such as antioxidant, antimicrobial and anti-inflammatory effects. Fatty acid methyl esters such as hexadecanoic acid methyl ester and 14,17-octadecanoic acid methyl ester were also identified. These compounds are commonly found in plant extracts and have been reported to exhibit potential pharmacological activities.

The GC-MS profile also revealed the presence of furan derivatives including furfural and 3-furaldehyde which are typical products of the Maillard reaction occurring during thermal processing. These compounds are known to contribute to the flavor and aroma characteristics of processed foods and have been reported to possess antioxidant properties.

Additionally, the heterocyclic compounds such as 4H-pyran-4-one derivatives were detected in the extract. These compounds have been reported to exhibit antioxidant and antimicrobial activities. The presence of such heterocyclic compounds further indicated that the aging process enhances the formation of functional phytochemicals in black garlic. Another compound detected in the analysis was diethyl phthalate which was occasionally been reported in plant extracts and may originate from environmental sources or laboratory materials used during the extraction.

Overall the GC -MS chromatographic profile (Figure 2) confirmed that the ethanolic extract of black garlic contains a diverse range of phytochemical compounds produced during the aging process. The presence of fatty acids, aldehydes, heterocyclic compounds and Maillard reaction products suggests that black garlic possess a complex phytochemical composition that may contribute to its reported antioxidant and hepatoprotective activities.

The identification of these compounds provides valuable information for further studies aimed at exploring the biological activities and therapeutic potential of black garlic particularly in relation to antioxidant and liver protective effects.

Conclusion

The present study characterized the phytochemical composition of the ethanolic extract of black garlic using GC-MS analysis. The chromatographic profiling revealed a diverse array of bioactive compounds belonging to multiple chemical classes including fatty acids, aldehydes, heterocyclic compounds, carbohydrate derivatives and esterified metabolites. Among the detected constituents' compounds such as n-hexadecanoic acid, oleic acid, 6-acetyl- β -D-mannose, furfural derivatives and several fatty acid methyl esters were identified indicating the formation of complex phytochemical metabolites during the thermal aging process of garlic. The presence of Maillard reaction products and lipid derived compounds confirms that controlled heat treatment significantly modifies the chemical composition of garlic, generating molecules associated with antioxidant and therapeutic properties.

Fatty acids and furan derivatives identified in the extract have been reported to exhibit antimicrobial, anti-inflammatory and antioxidant activities suggesting that these compounds may contribute to the biological functions attributed to black garlic.

Overall the GC-MS results demonstrate that the black garlic contains a rich spectrum of bioactive phytochemicals that may support its traditional use as a functional food with potential health benefits. These findings provide a chemical basis for further studies investigating the pharmacological mechanisms of black garlic and support further investigations involving molecular docking, in vitro assays and clinical evaluation to explore its hepatoprotective and antioxidant potential.

Figure 1: (A) Fresh garlic bulbs (B) Black garlic obtained after controlled aging under elevated temperature and humidity conditions showing the darkened cloves and characteristic soft texture formed during aging process

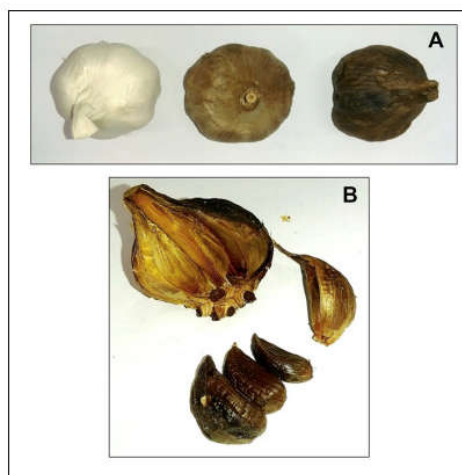
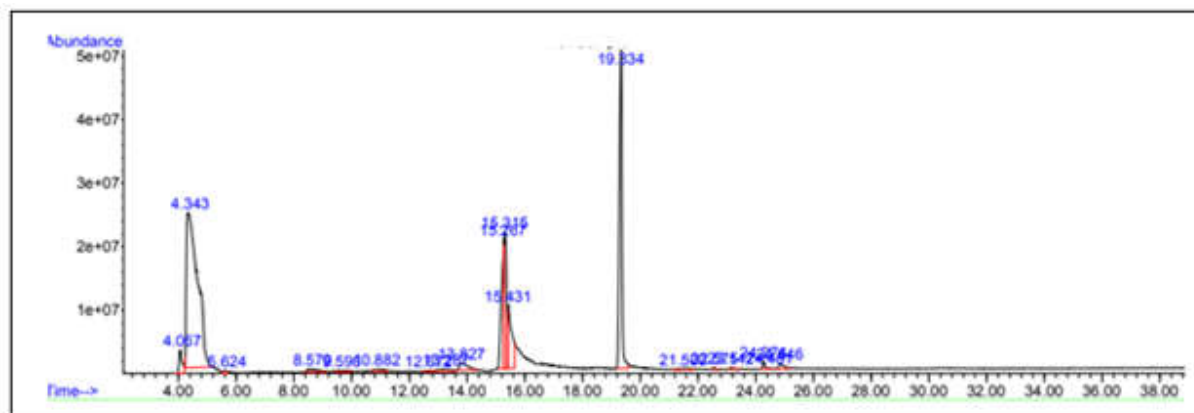


Figure 2: GC-MS chromatogram of the ethanolic extract of black garlic**Table 1: Phytochemical compounds identified in the ethanolic extract of black garlic using GC-MS.**

S.No	Compound Name	CID	Mol formula	Mol Wt	Exact Mass	NIST ID
1	n-Hexadecanoic acid	985	C16H32O2	256	256.24023	151973
2	Carbon dioxide	280	CO ₂	44	43.9898293	69
3	Ethanol	702	C ₂ H ₆ O	46	46.0418649	118507
4	5-Methyloxazolidine	536661	C ₄ H ₉ NO	87	87.0684137	280913
5	Furfural	7362	C ₅ H ₄ O ₂	96	96.021129	118785
6	3-Furaldehyde	10351	C ₅ H ₄ O ₂	96	96.021129	118098
7	6-Acetyl-β-D-mannose	—	C ₈ H ₁₄ O ₇	222	222.073953	130099
8	2,5-Dimethyl-4-hydroxy-3(2H)-furanone	19309	C ₆ H ₈ O ₃	128	128.047344	233718
9	3-Furancarboxylic acid, methyl ester	14918	C ₆ H ₆ O ₃	126	126.031694	332779
10	4H-Pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	119838	C ₆ H ₈ O ₄	144	144.042258	156511
11	5-Hydroxymethylfurfural	237332	C ₆ H ₆ O ₃	126	126.031694	231276

12	Diethyl phthalate	6781	C12H14O4	222	222.089209	341377
13	1-Isobutyl-7,7-dimethyl-octahydro-isobenzofuran-3a-ol	597971	C14H26O2	226	226.19328	189139
14	Hexadecanoic acid, methyl ester	8181	C17H34O2	270	270.25588	333716
15	14,17-Octadecadienoic acid, methyl ester	5365751	C19H34O2	294	294.25588	36085
16	Heptadecanoic acid, 16-methyl-, methyl ester	110444	C19H38O2	298	298.28718	23226
17	Oleic acid	445639	C18H34O2	282	282.25588	134027

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