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Research Article

Profiling of organosulfur compounds and amino acids in novel variety of *Allium sativum* (Hisar garlic 17) by HR-LCMS-QTOF

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Abstract
Over the past few years, the biological properties of garlic have been utilized as an attractive natural alternative to many therapeutic drugs. The biological effects of garlic have been ascribed to organosulfur compounds, secondary metabolites derived from amino acids. The present study aimed to investigate the extracts of a novel garlic variety (Hisar garlic 17) after processing it as fresh, dry, heated, and aged in different solvents and then analyzed with highly sensitive and rapid technique i.e., High resolution liquid chromatograph mass spectrometer quadrupole time of flight (HR-LCMS-QTOF) Mass spectrometer to study the amino acids and organosulfur compounds. 47 amino acids and 11 organosulfur compounds were detected out of which 8 organosulfur compounds were found as secondary metabolites of amino acids. Among the 22 crucial α-amino acids, garlic extracts revealed the presence of 18 amino acids, either in their native state or associated with various complex compounds. The study reported major organosulfur compounds, including Alliin, S-allyl cysteine, S-methyl cysteine, N-gamma-Glutamyl-S-allylcysteine, and 2-Propenyl 1-(2- propenylsulfinyl)propyl disulfide. This study demonstrated that HG17 garlic is abundant in amino acids and organosulfur compounds, suggesting its potential utilization as a supplement in nutraceuticals.

Keywords: Aged garlic, *Allium sativum*, Amino acids, Dry garlic, Fresh garlic, Heated garlic, Organosulfur compounds

INTRODUCTION

Garlic (*Allium sativum* L.) holds a significant position among the top twenty essential vegetables and is globally recognized as a crucial component in traditional medicinal practices worldwide (Ansary *et al.,* 2020). Originating from South Asia, Central Asia, and northeastern Iran, garlic is primarily cultivated in China, making it the leading global producer. In 2021, India secured the second position among the top 10 garlic-producing countries. Garlic is utilized in both its fresh state and various processed forms such as dry garlic, heated garlic, or aged garlic. Garlic is renowned for not just its rich nutritional content but also for its therapeutic and medicinal attributes, holds significance in both traditional and contemporary medical practices. Garlic has received growing attention for its health-promoting attributes, including antimicrobial properties, anticancer effects, potential in preventing and treating cardiovascular disease, and antioxidant capabilities (Gao *et al.,* 2020; Nakamoto *et al.,* 2020; De Greef *et al.,* 2021 and Farhat *et al.,* 2021).

Garlic composition has been widely studied and reviewed, placing particular emphasis on organosulfur compounds, which are primarily responsible for the numerous beneficial properties associated with garlic. The well-known and most abundant bioactive compounds found in garlic are alliin, ajoene's, allyl sulfides, 1,2-Vinyldithiin possessing antioxidant, antimicrobial, anticancer, antithrombotic, and cardioprotective properties (Kovarovič *et al.,* 2019; Espinoza *et al.,* 2020; Ruiz-Sánchez *et al.,* 2020 and Torres-Palazzolo *et al.,* 2020). Aside from organosulfur compounds that contribute to its flavor, amino acids serve as the primary precursors for the synthesis of cysteine sulfoxides and glutamyl peptides in garlic. Moreover, amino acids hold vital significance in the human body, functioning not only as fundamental building blocks for proteins and an energy source but also playing a crucial role in the biosynthesis of neurotransmitters, porphyrins, polyamines, and nitric oxides (Xiao and Guo, 2022).

The characteristics of garlic can be heightened through diverse treatments such as fermentation, heating, and controlled drying of garlic samples. Currently, a variant known as black garlic is gaining popularity. Black garlic, exhibiting significantly enhanced biological properties compared to fresh garlic, is produced through a thermal treatment process lasting 1-3 months at 60-80°C under controlled humidity conditions, without the use of additives. In these specified conditions, various chemical reactions including Maillard and enzymatic reactions, lead to a transformation in the color of garlic from white to black. This process results in a significant alteration of both composition and flavor (Ahmed and Wang, 2021). Black garlic exhibited anti-allergic activity, protective effect against diabetes, antihypertensive activity, increased function of immune cells, anti-hyperlipidemic, and anti-oxidative potential (Sembiring and Iskandar, 2019; Oktari *et al.,* 2020; Afzaal *et al.,* 2021; Ahmed and Wang, 2021; Saeed *et al.,* 2021 and Serrano *et al.,* 2023).

In recent years, the field of drug discovery has predominantly concentrated on exploring the remarkable biological properties of garlic and identifying novel bioactive compounds for therapeutic applications. Numerous published research studies in this context have focused on antioxidant potential and total polyphenol content. Additionally, other research has delved into changes within specific compound families following particular treatments. For instance, Bae *et al.* (2014) investigated alterations in S-allyl cysteine content before and after heat treatment, while Sato *et al.* (2006) examined tetrahydro-β-carboline derivatives. Liang *et al.* (2015) conducted a comparison between fresh and fermented garlic, analyzing seventeen amino acids, and two organosulfur compounds.

Garlic-based dietary supplements are available in diverse forms, including powdered capsules, oils, and dehydrated or lyophilized garlic. The use of analytical methods to identify active components is essential for gauging the nutraceutical value of garlic. In continuation of earlier studies, the present research seeks to evaluate and provide a comparative analysis of two crucial families of bioactive compounds (amino acids and organosulfur compounds) in HG17 garlic subjected to various processing methods.

MATERIALS AND METHODS

Plant material, Chemicals, and Standards

Garlic bulbs of variety Hisar garlic genotype 17 (HG17) were obtained from Department of Vegetable Science, Chaudhary Charan Singh Haryana Agriculture University (CCSHAU), Hisar (India). Garlic bulbs were completely sealed in airtight bags and followed by airtight containers at 4° Celsius. Solvents used for the extraction of garlic i.e., methanol, n-butanol, and ethyl acetate were of analytical grade and obtained from Merck (Mumbai, India). Milli-Q System (Merck, Mumbai, India) was used to make ultrapure water.

Preparation of different garlic extracts

Preparation of fresh garlic extract

Garlic cloves were peeled, cleaned and ground with the help of a mortar pestle and extracted with different solvents (50% methanol, n-butanol, and ethyl acetate) according to Fig. 1. Prepared samples were labelled as fresh garlic extract in 50% methanol solvent (FgM), fresh garlic extract in butanol solvent (FgB), and fresh garlic extract in ethyl acetate solvent (FgEA) (Bakht *et al.,* 2011).

Preparation of dried garlic powder extract

Garlic cloves were peeled and dried in shaded area for 3 months to obtain a constant dried weight and then pulverized with the aid of electric mixer. One gram of dry garlic powder was calculated equal to 4.6 g fresh garlic weight. Then dry sample of garlic was extracted with different solvents (50% methanol and butanol) according to Fig. 1 and labelled as dry garlic extract in 50% methanol solvent (DgM), dry garlic extract in butanol solvent (DgB) (Saha and Bandyopadhyay, 2017).

Preparation of heated garlic extract

Garlic bulbs were sheathed in aluminium foil, then kept in a steel container, and set in oven at 130°C for 2 hours. Garlic cloves were peeled, grinded, and extracted by shaking at room temperature with different solvents (50% methanol and butanol) as shown in Fig. 1. Prepared extracts were labelled as heated garlic extract in 50% methanol solvent (HgM), heated garlic extract in butanol solvent (HgB) (Yun *et al.,* 2016).

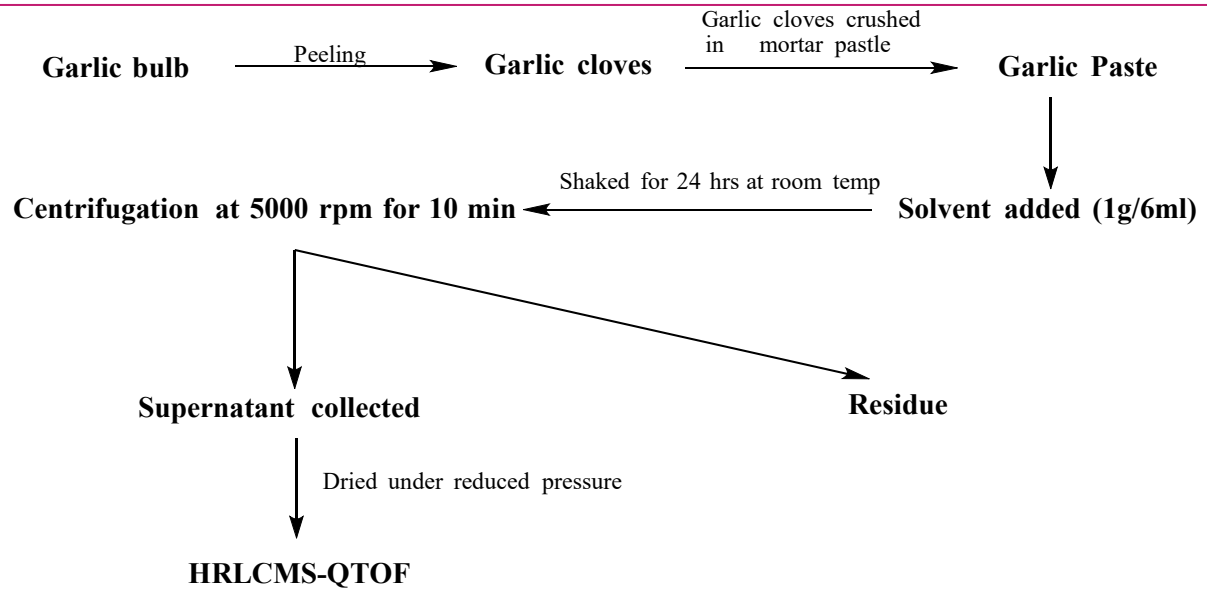

Fig. 1. *Preparation of different garlic extracts*

Preparation of aged garlic extract

Garlic bulbs were enveloped in aluminum foil, kept in a steel container, and then positioned in humidity chamber for 15 days at 60˚C with 80% humidity. This procedure was performed at CSIR-Central Road Research Institute, New-Delhi, India. Garlic cloves were cleaned, peeled, and extracted with different solvents (50% methanol and butanol) according to **Fig. 1**. Prepared samples were labelled as aged garlic extract in 50% methanol solvent (AgM), aged garlic extract in butanol solvent (AgB) (Zhang *et al.,* 2016).

HR-LCMS-QTOF analysis

All the prepared extracts were further subjected to rotary evaporator and crude extracts were sent to Sophisticated Analytical Instrument Facility, Indian Institute of Technology (SAIF-IIT), Mumbai for analysis. High resolution liquid chromatograph mass spectrometer (HR-LCMS) was done by adjusting its scanning scale of 120 -1200 m/z for MS/MS. The system consisted of a HiP sampler, binary gradient solvent pump, column compartment and Quadrupole Time of Flight Mass Spectrometer (MS Q-TOF). The analysis utilized a 5 µl sample injection, followed by Hypersil GOLD C18 Column measuring 100 mm×2.1 mm×3 microns for separation. 0.1% formic acid in water and acetonitrile were used as solvent A and B respectively with 0.300 mL/min flow rate in binary pump. The following settings were applied: Gas flow of 13 L/min, Gas Temperature at 250˚C, Nebulizer pressure set to 35 psig, Sheath gas flow at 11, Sheath gas temperature at 300˚C, Fragmentor set to 175, Skimmer1 at 65, VCap at 3500, Nozzle Voltage at 1000 V, Octopole RF Peak at 750. For auxiliary specifications, Draw Position Offset was 0.0 mm, Draw Speed and Eject Speed were both 100.0 µL/min, Vial/Well bottom sensing was enabled, Sample Flush Out Factor set to 5.0, and a 2.0 s hold time was applied after drawing (Noumi *et al.,* 2020).

RESULTS AND DISCUSSION

Bioactive compounds in fresh, dry, aged and thermally processed garlic (*Allium sativum* L.)

In this study, 47 amino acids (Table 1) and 11 organosulfur compounds (Table 3) were identified and characterized by HR-LCMS-QTOF technique. HR-LCMS-MS zoomed spectrum showing the ionization peaks of organosulfur compounds and amino acids detected in various extracts of garlic, with their chemical formula and structure have been provided as Fig, S1 and S2 in the supplementary files.

In our study, different garlic extracts revealed the presence of 19 α-amino acids (Cys, Ser, Thr, Ile, Ala, Arg, Pro, Gln, Glu, His, Gly, Leu, Lys, Asn, Asp, Trp, Met & Phe), either in their native state or associated with various complex compounds. Our study reported presence of various organosulfur compounds, including S-allyl-L-cysteine, N gamma glutamyl-allylcysteine, L-gamma-glutamyl-(s)-allylthio-l-cysteine, gamma-Glutamyl-s-methylcysteine, Alliin, (+/-)-3-[(2-methyl-3-furyl)thio]-2-butanone, Benzo[b]naphtho[2,1-d]thiophene, S-Methyl-L-cysteine, N-gamma-Glutamyl-S-trans-(1-propenyl) cysteine, (gamma-Glutamyl-gamma-glutamyl)-S-methylcysteine & 2-Propenyl 1-(2- propenylsulfinyl) propyl disulfide in HG17.

Liu *et al.* (2020) study revealed the existence of total 7 organosulfur compounds and 20 amino acids in 242 samples of garlic from china. Interestingly, the numbers reported in their study were notably fewer than those observed in our research. Specifically, Liu *et al.* (2020) reported S-allyl-L-cysteine, N gamma glutamyl-

allylcysteine, gamma-Glutamyl-s-methylcysteine, Alliin, S-Methyl-L-cysteine which were similar to our findings. However, there were disparities between the two studies, as methiin and allicin, noted by Liu *et al.* (2020) were absent in our investigation. Instead, our study unveiled the presence of six distinct organosulfur compounds (O3, O6, O7, O9, O10, O11). This discrepancy highlights the variability in garlic samples and underscores the importance of comprehensive analysis to detect the diverse composition of these compounds in garlic.

Comparison of amino acids between fresh garlic and processed garlic (dry, heated, and aged variations)

Amino acids are the building blocks of proteins, contribute to vital biological processes such as protein synthesis, enzyme activity, and cell signaling, playing essential roles in the structure and function of living organisms. Their diverse functions encompass energy production, immune support, and neurotransmitter synthesis, highlighting their critical importance in maintaining overall health.

In our study, total 47 amino acids were identified in different extracts of garlic processed by various methods. Lee and Harnly (2005) reported 28 amino acids in 11 garlic samples which were lower than our findings. They combinedly reported presence of alliin, alanine, sarcosine, glycine, R-aminobutyric acid, valine, leucine, allo-isoleucine, isoleucine, threonine, serine, proline, asparagine, aspartic acid, methionine, 4-hydroxyproline, glutamic acid, phenylalanine, R-aminoadipic acid, glutamine, ornithine, lysine, histidine, tyrosine, tryptophan, cysteine by GCMS. Kim *et al.,* (2012) reported 17 amino acids (Alanine, Arginine, Aspartate, Cysteine, Glutamate, Glycine, Histidine, Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Proline, Serine, Threonine, Tyrosine, Valine) in raw garlic by HPLC. Our data is fully validated with previous studies and revealed almost similar amino acids in HG17 extracts with the addition of few different amino acids. Histidine, Arginine, & Tryptophan were detected in their native structure, and the rest were detected as derivatives of amino acids. Histidine and tryptophan are two essential amino acids and tryptophan is the largest proteinogenic amino acid. Table 1 shows the availability status of different amino acids in various extracts of garlic. Table 2 shows molecular weight, molecular formula and retention time of amino acids found in various extracts of garlic.

6 amino acids (A1, A3, A4, A9, A44 & A46) were detected in each extract. Arginine & Tryptophan were the major α-amino acids, and L-threo-3-Phenyl Serine & N-acetyldjenkolic acid were derivatives of amino acids detected in all extracts. In fresh garlic, 27 amino acids were detected. After drying (A2, A5, A10, A11, A15, A19, A20, A23, A25, A27, A28, A33, A36, A37, A45, A47) amino acids disappeared which were present in fresh garlic. A14, A16, A39, A42 were newly formed amino acids after drying treatment which were absent in fresh garlic.

After aging 17 amino acids (A6, A7, A8, A10, A11, A15, A19, A20, A23, A25, A27, A28, A33, A36, A37, A45 & A47) which were present in fresh garlic disappeared but aging led to the formation of 13 new amino acids (A14, A17, A18, A22, A24, A26, A30, A32, A35, A38, A40, A41 & A43). One amino acid derivative was exclusively detected in black garlic i.e., norharman, formed by condensation of tryptophan with pyruvic acid, thus suggesting that tryptophan reacts during garlic heat treatment to form norharman (Molina-Calle *et al.,* 2017). Similarly, Harman and norharman were only detected in aged extract in our study. Asn, Ala, Ser, Thr, Glu, Asp, Pro, Arg, Phe, Orn, Lys, and Tyr, were observed in the methanol extract of black garlic (Onozato *et al.,* 2023). In our study, aged extract has all these amino acids except Asn, Ala, Orn, Lys, Tyr, Thr but our extract has His, Trp, Ile which were absent in reported study. The sulfur-containing cysteine content of black garlic decreased significantly during aging (Choi *et al.,* 2014). The total number of amino acids found in dry and aged treatment of fresh garlic were 15 and 23 respectively. Prolyl-Arginine was the new compound found in both dry & aged treatment of fresh garlic samples and known for antibacterial properties (Sepahi *et al.,* 2017). Cephalosporin C was found in aged treatment of fresh garlic and known for remarkable antimicrobial activity (Beatriz *et al.,* 2022).

In comparison of fresh garlic with heated extract, it was observed that total 15 amino acids were the same. After heating 12 amino acids (A11, A12, A13, A15, A19, A20, A25, A27, A33, A36, A37 & A47) disappeared which were present in fresh garlic and 3 new amino acids (A21, A31, A34) appeared. This happened because of Maillard reaction: a chemical reaction between amino acids and reducing sugars. On the treatment of heat, Prenyl-L-cysteine was the newly formed compound which is responsible for immunomodulatory effects *in vitro* and *in vivo*, and reduced blood pressure in a hypertensive animal model (Kodera *et al.,* 2017). All these results indicated that processing and area of cultivation has direct effect on compounds.

Comparison of organosulfur compounds between fresh garlic and processed garlic (dry, heated, and aged variations)

In present study, total 11 organosulfur compounds were detected in different extracts of garlic (Table 3 and Table 4). The organosulfur compounds in garlic are well known for their anti-inflammatory, antithrombotic, and anti-cancerous biological activities (Shang *et al.,* 2019). Table 3 shows the availability status of different organo-

Table 1. Total amino acids found in various extract's i.e., Fresh garlic extract (FG), Dry garlic extract (DG), Aged garlic extract (AG), Heated garlic extract (HG); Zero (0) indicate the absence and one (1) indicates the presence of amino acid in the respective extract

Sr. No.	Compound name	FG	DG	AG	HG
A1	N-gamma-Glutamyl-S-allylcysteine	1	1	1	1
A2	L-gamma-Glutamyl-S-allylthio- L-cysteine	1	0	1	1
A3	N-Acetyldjenkolic acid	1	1	1	1
A4	L-threo-3-Phenylserine	1	1	1	1
A5	N-D-Glucosylarylamine	1	0	1	1
A6	S-Allyl-L-cysteine	1	1	0	1
A7	gamma-Glutamyl-S-methylcysteine	1	1	0	1
A8	4-Hydroxy-L-threonine	1	1	0	1
A9	Alliin	1	1	1	1
A10	Ustiloxin C	1	0	0	1
A11	Glutamyl-isoleucine	1	0	0	0
A12	N-(1-Deoxy-1-fructosyl)phenylalanine	1	1	1	0
A13	N2-Fructopyranosylarginine	1	1	1	0
A14	Prolyl-Arginine	0	1	1	0
A15	3-Methylthiopropanamine	1	0	0	0
A16	5-Methyltetrahydropteroyltri-L-glutamate	0	1	0	0
A17	Cephalosporin C	0	0	1	0
A18	Glutaminyl-Histidine	0	0	1	0
A19	Histidinyl-Proline	1	0	0	0
A20	L-prolyl-L-glycine	1	0	0	0
A21	Prenyl-L-cysteine	0	0	0	1
A22	S-Prenyl-L-cysteine	0	0	1	0
A23	Glutamyl-leucine	1	0	0	1
A24	Harman	0	0	1	0
A25	Jadomycin A	1	0	0	0
A26	Norharman	0	0	1	0
A27	N5-(4-Methoxybenzyl)glutamine	1	0	0	0
A28	Nα-Acetyl-L-arginine	1	0	0	0
A29	(gamma-Glutamyl-gamma-glutamyl)-S-methylcysteine	0	0	0	1
A30	Aralionine A	0	0	1	0
A31	S-Methyl-L-cysteine	0	0	0	1
A32	s-phenylmercapturic acid	0	0	1	0
A33	Histidinyl-Asparagine	1	0	0	0
A34	L-Pyrrolysine	0	0	0	1
A35	Methionyl-Aspartate	0	0	1	0
A36	N-gamma-Glutamyl-S-trans-(1-propenyl)cysteine	1	0	0	0
A37	Phenylbutyrylglutamine	1	0	0	0
A38	Tryptophyl-Isoleucine	0	0	1	0
A39	Lathyrine	0	1	0	0
A40	Musca-aurin-I	0	0	1	0
A41	Myriocin	0	0	1	0
A42	Vulgaxanthin-I	0	1	0	0
A43	Histidine	0	0	1	0
A44	L-Tryptophan	1	1	1	1
A45	L-Arginine	1	0	0	1
A46	D-Arginine	1	1	1	1
A47	delta-Guanidinovaleric acid	1	0	0	0

sulfur compounds in various extracts of garlic. Table 4 shows molecular weight, molecular formula and retention time of organosulfur compounds found in various extracts of garlic.

3 organosulfur compounds (S-allyl cysteine, N-gamma glutamyl-allyl cysteine and Alliin) were commonly detected in fresh as well as processed garlic samples. This indicated that these three are most stable and major organosulfur compounds present in garlic. The present study supported the facts that alliin is one of the most important secondary metabolites present in garlic and S-allyl cysteine is the water soluble, most bioactive compound known in garlic (Wang *et al.,* 2017; Yudhistira *et al.,* 2022).

Out of 11 organosulfur compounds, eight compounds (O1, O2, O3, O4, O5, O6, O9 & O11) were detected in fresh garlic extract. Upon heating, the composition changed, and 9 organosulfur compounds (O1, O2, O3,

Table 2. Mass and retention time of amino acids found in various extract's i.e., Fresh garlic extract (FG), Dry garlic extract (DG), Aged garlic extract (AG), Heated garlic extract (HG); Zero (0) indicate the absence.

Sr. No.	Mol. Formula	Mass	M/z	FG (RT)	DG (RT)	AG (RT)	HG (RT)
A1	C11H18N2O5S	290.0932	291.1005	4.692	3.155	8.144	9.677
A2	C11H18N2O5S2	322..0642	323.0714	6.013	0	9.068	10.948
A3	C9H16N2O5S2	296.0492	297.0565	4.548	2.835	7.493	7.853
A4	C9H11NO3	181.0735	182.0808	1.877	1.382	2.131	2.233
A5	C12H17NO5	255.1121	278.1013	5.456	0	8.727	10.584
A6	C6H11NO2S	161.0504	162.0576	4.776	3.209	0	9.732
A7	C9H16N2O5S	264.0775	265.0848	2.009	1.433	0	2.302
A8	C4H9NO4	135.0538	136.0611	4.086	1.528	0	2.57
A9	C6H11NO3S	177.0455	178.0527	1.362	1.164	1.397	1.308
A10	C23H34N4O10S	558.2027	581.1923	4.797	0	0	9.81
A11	C11H20N2O5	260.1361	261.1434	5.035	0	0	0
A12	C15H21NO7	327.1307	328.1381	0	2.181	5.624	0
A13	C12H24N4O7	336.1637	337.1709	0.93	0.919	1.19	0
A14	C11H21N5O3	271.1646	294.1537	0	1.545	2.49	0
A15	C4H11NS	105.06	164.0739	0	4.389	0	0
A16	C25H36N8O12	640.2494	639.2421	0	11.075	0	0
A17	C16H21N3O8S	415.1032	460.1013	0	0	1.635	0
A18	C11H17N5O4	283.1265	282.119	0	0	11.096	0
A19	C11H16N4O3	252.1244	297.1226	4.399	0	0	0
A20	C7H12N2O3	172.0869	171.0796	8.783	0	0	0
A21	C8H15NO2S	189.0825	248.0962	0	0	0	11.015
A22	C8H15NO2S	189.0837	248.0974	0	0	10.468	0
A23	C11H20N2O5	260.1356	261.1428	5.315	0	0	10.093
A24	C12H10N2	182.0836	183.0909	0	0	9.234	0
A25	C24H21NO6	419.137	420.1437	5.6	0	0	0
A26	C11H8N2	168.0679	169.0751	0	0	9.152	0
A27	C13H18N2O4	266.1261	267.1332	4.622	0	0	0
A28	C8H16N4O3	216.1217	217.1289	1.419	0	0	0
A29	C14H23N3O8S	393.1202	394.1274	0	0	0	9.216
A30	C34H38N4O5	582.2955	314.137	0	0	8.228	0
A31	C4H9NO2S	135.0353	136.0426	0	0	0	2.373
A32	C11H13NO3S	239.0653	262.0544	0	0	6.076	0
A33	C10H15N5O4	269.112	292.1022	5.892	0	0	0
A34	C12H21N3O3	255.157	256.1643	0	0	0	10.883
A35	C9H16N205S	264.0769	265.0841	0	0	2.334	0
A36	C11H18N2O5S	290.0929	313.0821	5.449	0	0	0
A37	C15H20N2O4	292.1396	315.1288	3.389	0	0	0
A38	C17H23N3O3	317.1719	318.1793	0	0	9.307	0
A39	C7H10N4O2	182.0807	181.0733	0	1.095	0	0
A40	C14H13N3O8	351.0679	350.0609	0	0	6.348	0
A41	C21H39NO6	401.2851	400.2778	0	0	11.985	0
A42	C14H17N3O7	339.1034	338.0961	0	1.606	0	0
A43	C6H9N3O2	155.0712	154.0638	0	0	1.071	0
A44	C11H12N2O2	204.0896	205.0967	3.227	3.46	4.621	9.386
A45	C6H14N4O2	174.1129	173.1056	1.094	0	0	1.054
A46	C6H14N4O2	174.1113	175.1185	0.946	0.913	1.037	1.141
A47	C6H13N3O2	159.1003	160.1075	1.88	0	0	0

O4, O5, O6, O7, O8, & O10) were detected. Notably, N-gamma-Glutamyl-S-trans-(1-propenyl) cysteine and 2-Propenyl 1-(2-propenylsulfinyl)propyl disulfide, identified in the fresh garlic, were no longer present in the heated sample. This observation aligns with findings from Molina-Calle *et al.* (2017), who also reported the

Table 3. Total organosulfur found in various extract's i.e., Fresh garlic extract (FG), Dry garlic extract (DG), Aged garlic extract (AG), Heated garlic extract (HG); Zero (0) indicate the absence and one (1) indicates the presence of organosulfur in the respective extract

Sr. No.	Compound	FG	HG	AG	DG
O1	S-allyl-L-cysteine	1	1	1	1
O2	N gamma glutamyl-allylcysteine	1	1	1	1
O3	L-gamma-glutamyl-(s)-allylthio-l-cysteine	1	1	1	0
O4	gamma-Glutamyl-s-methylcysteine	1	1	0	1
O5	Alliin	1	1	1	1
O6	(+/-)-3-[(2-methyl-3-furyl)thio]-2-butanone	1	1	0	0
O7	Benzo[b]naphtho[2,1-d]thiophene	0	1	0	0
O8	S-Methyl-L-cysteine	0	1	0	0
O9	N-gamma-Glutamyl-S-trans-(1-propenyl)cysteine	1	0	0	0
O10	(gamma-Glutamyl-gamma-glutamyl)-S-methylcysteine	0	1	0	0
O11	2-Propenyl 1-(2- propenylsulfinyl)propyl disulfide	1	0	0	0

Table 4. Mass and retention time of organosulphur found in various extract's i.e., Fresh garlic extract (FG), Dry garlic extract (DG), Aged garlic extract (AG), Heated garlic extract (HG), Zero (0) indicate the absence

Sr. No.	Mol. Formula	Mass	M/z (M+H)+	FG (RT)	DG (RT)	AG (RT)	HG (RT)
O1	C6H11NO2S	161.0504	162.0576	4.776	3.209	0	9.732
O2	C11H18N2O5S	290.0932	291.1005	4.692	3.155	8.144	9.677
O3	C11H18N2O5S2	322.0642	323.0714	6.013	0	9.068	10.948
O4	C9H16N2O5S	264.0775	265.0848	2.009	1.433	0	2.302
O5	C6H11NO3S	177.0455	178.0527	1.362	1.164	1.397	1.308
O6	C9H12O2S	184.0582	185.0655	0.814	0	0	0.986
O7	C6H10S	234.0486	235.056	1.022	0	0	1.147
O8	C4H9NO2S	135.0353	136.0426	0	0	0	2.373
O9	C11H18N2O5S	290.0929	313.0821	5.449	0	0	0
O10	C14H23N3O8S	393.1202	394.1274	0	0	0	9.216
O11	C9H16OS3	236.0353	237.0425	9.471	0	0	0

disappearance of γ-glutamyl-S-(1-propenyl)-cysteine in fresh garlic after heating. Molina-Calle *et al.* (2017) highlighted the inherent instability of thiosulfinates, and their replacement by various sulfur volatiles in garlic. In this context, they reported the disappearance of diallyl disulfide in heated garlic similar to our study (Molina-Calle *et al.,* 2017).

Upon subjecting garlic to heat treatment, the emergence of Benzo[b]naphtho[2,1-d]thiophene, S-Methyl-L-cysteine, and (gamma-Glutamyl-gamma-glutamyl)-S-methylcysteine compounds was observed, which were absent in fresh garlic. This suggests that the heating process induces the formation of distinct organosulfur compounds beyond the basic ones. S-Methyl-L-cysteine and (gamma-Glutamyl-gamma-glutamyl)-S-methylcysteine, recognized for their capacity to enhance anti-inflammatory properties and address insulin resistance (Thomas *et al.,* 2015), are among the newly identified compounds. Benzo[b]naphtho[2,1-d] thiophene, known for its potent antimicrobial properties (Algso *et al.,* 2018), is also present. The heat-induced transformation is due to pyrolysis, which lead to the removal of lower hydrocarbon groups from complex compounds. This process potentially enhances the biological properties of garlic in specific directions (Kang, 2016).

In dry garlic, only 4 organosulfur compounds (O1, O2, O4 & O5) detected. O3, O6, O9 & O11 which were present in fresh garlic disappeared after drying process. After aging treatment of 15 days, only O1, O2, O3 & O5 detected in aged samples. The outcomes of both drying and aging treatments revealed a reduction in the number of organosulfur compounds found in fresh garlic, indicating that prolonged storage diminishes their presence. N-gamma-Glutamyl-S-trans-(1-propenyl)cysteine and 2-Propenyl 1-(2-propenylsulfinyl)propyl disulfide were exclusively identified in fresh garlic, underscoring their high instability, susceptible to destruction through any form of processing. Organosulfur compounds reported in the aqueous and alcoholic extract of garlic include S-allyl cysteine (SAC), S-allylmercapto-L-cysteine (SAMC), and S-methyl cysteine (Bhatwalkar *et al.,* 2021). Notably, γ-glutamyl-S-methyl-L-cysteine and γ-glutamyl-S-(1-propenyl)-L-cysteine were previously considered exclusive to black garlic (Molina-Calle *et al.,* 2017), but our study identified these two compounds in fresh garlic.

In Iraqi garlic, Al-Taai *et al.* (2019) reported sulfur-containing compounds, such as diallyl disulphide and di -2-propenyl tetra sulfide, using GCMS and HPLC in

petroleum ether solvent. Conversely, another study highlighted the presence of S-allyl cysteine in black (aged) garlic extract using HPLC-UVD and HPLC-FLD (Bae *et al.,* 2012). Various sulfur-containing compounds found in garlic, including allicin, alliin, ajoenes, and vinyldithiins, have been documented in previous studies (El-Saber Batiha *et al.,* 2020; Liu *et al.,* 2020; Naznin *et al.,* 2008). Allicin, a lipid-soluble compound, easily degrades into metabolites like alliin, ajoenes, and vinyldithiins. Ajoenes and vinyldithiins are specifically isolatable from garlic in oil form or chloroformic extracts (El-Saber Batiha *et al.,* 2020). Our study aligns with existing research by confirming the presence of sulfur-containing compounds, including disulphide, SAC, Alliin, etc., in various extracts of HG17.

Conclusion

Garlic (*Allium sativum*) stands as one of the earliest cultivated plants. In this study, we identified 11 organosulfur compounds and 19 α-amino acids in a specific garlic variety i.e., Hisar Garlic 17. Additionally, we investigated the impact of various processing methods on the composition of bioactive compounds in garlic samples. The application of heat or the aging process in garlic trigger chemical reactions that result in the formation of diverse amino acids and organosulfur compounds compared to those found in fresh garlic. Considering the significant benefits associated with amino acids and organosulfur compounds, this particular garlic variety, HG17, holds potential for in-depth exploration in therapeutic applications. It could be utilized as a nutraceutical, offering a rich source of amino acids for supplementation.

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Supplementary information

The information regarding HR-LCMS-MS zoomed spectrum showing the ionization peaks of organosulfur compounds and amino acids detected in various extracts of garlic, with their chemical formula and structure have been provided as Fig. S1 and Fig. S2 in supplementary file. The author(s) is responsible for the content or functionality of any supplementary information. Any queries regarding the same should be directed to the corresponding author. The supplementary information is downloadable from the article's webpage and will not be printed in the print copy.

Conflict of interest

The authors declare that they have no conflict of interest.

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