

Analytical, Nutritional and Clinical Methods

Quantitative analysis of garlic (*Allium sativum*) oil unsaturated acyclic components using FT-Raman spectroscopy

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Abstract

A group of acyclic, unsaturated, sulfur containing, components present in garlic oil are quantitatively analyzed with FT-Raman spectroscopy. This group of components that may consist up to more than 70% of the garlic oil is responsible for some of the medicinal properties that are widely researched by scientists around the world. FT-Raman spectroscopy is evaluated against a gas-chromatography method. Band intensities at 1636 and 1606 cm^{-1} are used from untreated garlic oil spectra to determine the % content in acyclic, unsaturated sulfur containing compounds. The analyzed garlic oils were found to contain from 33.3% up to 75.3% of the targeted components. The proposed technique is simple, rapid and non-destructive for the sample.

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1. Introduction

Garlic (*Allium sativum*) has been famous for its flavor and aroma as well as for its medicinal uses for centuries (Agarwal, 1996; Vitranen, 1962). Today, exploitation and understanding of all of the advantages of garlic in nutrition and medicine is still in progress. It is generally accepted that the volatile compounds of garlic are the main reason for its properties (Block, 1992).

It was in 1892, when a German chemist, by the name of W. Semmler, reported that garlic cloves could be steam distilled to produce strong smelling essential oil. The characteristic odor of this essential oil is mainly caused by the presence of sulfur containing volatiles (Block, 1985).

More detailed analysis of garlic's volatiles has thereafter determined the existence of mono to hexa diallyl sulfides and vinyl dithiin isomers as the major constitu-

ents of garlic oil (Block, 1992). The medicinal and bioactive properties of the garlic oil are connected to the unsaturated acyclic components (Banerjee, Mukherjee, & Maulic, 2000; Benkeblia, 2004; Ho, Koh, Ma, Huang, & Sim, 1996). The main unsaturated acyclic and cyclic components are shown in Fig. 1.

In order to clarify garlic's odor, various isolation techniques have been used. Among them simple solvent extraction of raw garlic homogenate by organic solvents (Brodnitz, Pscale, & Van Derslice, 1971), aged garlic extracts (Dillon, Burmi, Lowe, Billington, & Rahman, 2003) and oil macerated garlic (Lawson, 1991). Also distillation setups such as simultaneous distillation extraction (SDE) (Yu, Wu, & Liou, 1989) and microwave-assisted hydrodistillation (MWHd) is been used (experimental data from our team have been submitted for publication). Experimental data from these techniques revealed the existence of highly reactive and thermally unstable organosulfur compounds. These molecules take part in spontaneous reactions that are very difficult to control (Block, 1992).

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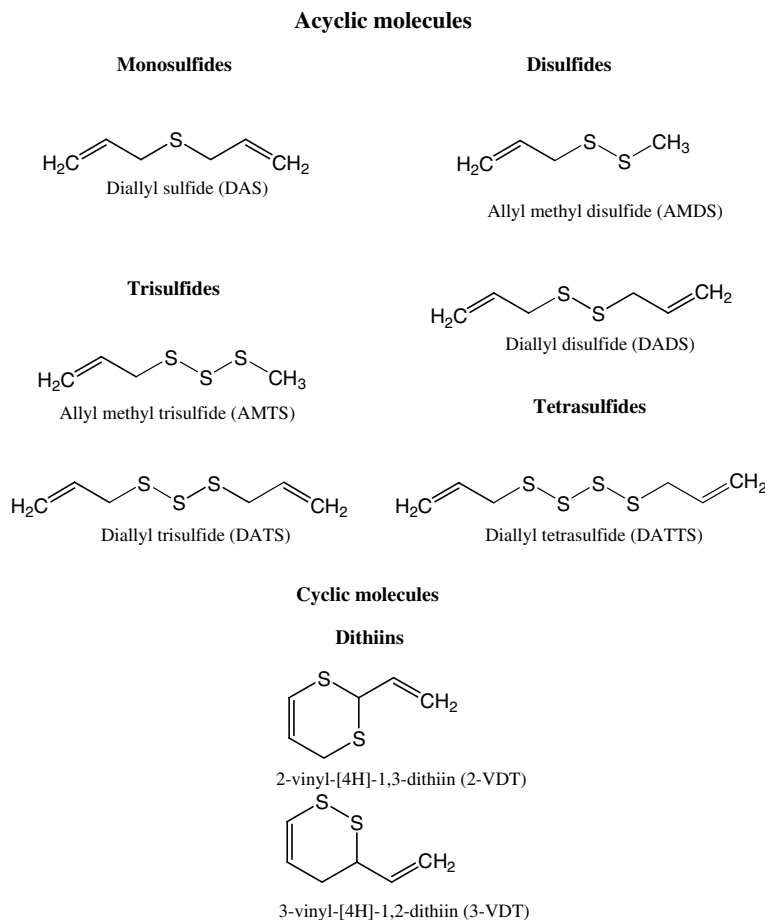


Fig. 1. The molecular structures of the main unsaturated acyclic and cyclic components of garlic oil.

The analytical methods for the qualitative and quantitative determination of garlic's composition are traditionally GC-MS, GC-FID, HPLC, and UV. Due to the unstable nature of such compounds, certain improvements on isolation and analytical methods to minimize the effect of heat are necessary.

The improvements proposed so far, regard the use of ultrasound-assisted extraction (USE) at room or even lower temperatures (experimental data from our team have been submitted for publication), and more sophisticated "cryogenic" GC-MS for the application of low temperatures during the analysis (Abu-Lafi, Dembitsky, Goldshlag, Hanus, & Dembitsky, 2004).

Taking into account the particular requirements of the studied sample, Raman spectroscopy appears to be a very promising technique. Indeed, FT-Raman spectroscopy is an analytical technique based on the interaction of an incident monochromatic radiation with vibrational energy levels of molecules. It is widely used for qualitative comparisons between molecular species of various samples. The concentration of a compound in a mixture may be related to the Raman intensities if an appropriate reference material is used to determine its value (Daferera, Pappas, Tarantilis, & Polissiou,

2002; Hancewicz & Petty, 1995; Skoulika, Georgiou, & Polissiou, 2000; Sun, Ibrahim, Oldam, Schultz, & Conners, 1997). The analysis time is very short and variation due to analysis temperature (GC) or solvent (HPLC) minimized.

This paper describes the development of a method for quantitative analysis of acyclic components in garlic oil by FT-Raman spectroscopy. The recorded FT-Raman spectra of the garlic oils, which were collected using simultaneous distillation solvent extraction or microwave-assisted hydrodistillation extraction or ultrasound-assisted extraction, are treated in order to measure the compositions in acyclic compounds of analyzed oils. The proposed method is simple and rapid.

2. Materials and methods

2.1. Materials

2.1.1. Plant material and reagents

Garlic (*Allium sativum*) cloves were purchased from local farmers of N.Vissa a village near the North-East

borders of Greece. During the experiments, raw material was maintained in our lab with the appropriate conditions (dark, 25 °C). The garlic was milled and cut using a Moulinex cuisine blender. The used solvents were diethyl ether and ethyl acetate (purity 99.5%) supplied by BDH (Poole, England) and MERC (Darmstadt, Germany), respectively. Diallyl sulfide used as standard was purchased by ACROS ORGANICS (New Jersey, USA).

2.1.2. Preparation of standard samples

Garlic oil which was extracted by diethyl ether using ultrasounds contained the lowest percentage in acyclic compounds as it determined by GC-FID (12.7% v/v) (Table 1). Ten standards (st1–st10) were prepared by mixing appropriate amounts of the above garlic oil and diallyl sulfide. So the additional amounts in unsaturated acyclic components of standards were 16.7, 26.6, 37.5, 41.2, 47.7, 50.0, 54.5, 61.5, 66.7 and 76.2% v/v.

Table 1
Chemical composition (% v/v) of garlic (*Allium sativum*)

Peak no.	Compound	s1 ^a	s2 ^a	s3 ^b	s4 ^b	s5 ^c	s6 ^c
1	Dimethyl disulfide (DMS)	2.2	1.9	2.1	0.7		
2	Ethyl vinyl sulfide (EVS)					3.2	9.7
3	1,2-dithiacyclopentane	0.4	0.3	0.4	0.2	0.2	
4	Diallyl sulfide (DS)	2.3	1.9	0.9	0.1	0.3	0.4
5	2-Vinyl thiophene				0.2		
6	3-methylthio propanal	0.3	0.4		0.2		
7	Methyl allyl disulfide (cis or trans) (MADS)	9.1	8.5	9.0	4.3	0.6	1.4
8	Methyl propyl disulfide	0.1	0.1	0.1	0.2		
9	Methyl allyl disulfide (cis or trans) (MADS)	0.2	0.2	0.1	0.2		
10	Unknown (Yu et al)	2.4	1.6	1.8	1.5	0.7	2.0
11	Dimethyl trisulfide (DMTS)	2.0	2.3	2.7	2.0		
12	Allyl 2,3-epoxypropylsulfide			0.1		0.1	
13	Diallyl disulfide (DADS)	28.4	23.1	17.6	6.8	8.2	6.0
14	C ₆ H ₁₀ S ₂	0.5	0.4	0.3	0.5	0.1	
15	C ₆ H ₁₀ S ₂	1.2	0.9	1.4	1.1	1.5	3.7
16	Methyl butyl trisulfide				0.1		
17	Methyl allyl trisulfide (MATS)	16.3	17.5	14.1	14.9	0.1	0.2
18	3-Vinyl-[4H]-1,2-dithiin (3-VDT)	4.0	4.5	15.9	14.5	32.7	25.7
19	2-Vinyl-[4H]-1,3-dithiin (2-VDT)	5.5	8.7	17.7	27.7	38.1	34.4
20	1,4-dimethyl tetrasulfide (DMTTS)	0.4	0.4				
21	Diallyl trisulfide (DATS)	20.4	22.1	9.1	14.1	0.2	0.7
22	3,5-diethyl 1,2,4-trithiolane	0.2	0.2	0.2	0.2		0.5
23	Pentadecene						0.6
24	Heptadecene						0.5
25	3-(2-thia-4-pentenyl)-1-thiacyclohex-5-ene						0.3
26	Diallyl tetrasulfide (DATTS)	0.7	0.6	0.1	0.4		
27	3-(2,3,4-trithia-5-heptenyl)-1-thia-cyclohex-5-ene	0.1		0.2	0.2	0.1	
28	2-(1-thia-2-cyclohexen-6-yl)-1,3-dithia-cyclohex-5-ene	0.3	0.4	2.0	2.7	6.4	2.2
	Unidentified	3.0	4.0	4.2	7.2	7.5	11.7
	<i>Total unsaturated acyclic compounds content</i>	<i>77.4</i>	<i>73.9</i>	<i>51.0</i>	<i>41.0</i>	<i>12.7</i>	<i>19.5</i>

Volatile fractions obtained by three different extraction techniques (SDE, MWHD, USE) using diethyl ether and ethyl acetate.

^a s1 and s2 presents the percentage concentration of garlic's essential oil isolated by the SDE technique using diethyl ether and ethyl acetate, respectively.

^b s3 and s4 presents the percentage concentration of garlic's essential oil isolated by the MWHD technique using diethyl ether and ethyl acetate, respectively.

^c s5 and s6 presents the percentage concentration of garlic's essential oil isolated by the USE technique using diethyl ether and ethyl acetate, respectively.

2.2. Simultaneous distillation solvent extraction (SDE)

Simultaneous distillation extraction was carried out on Lickens-Nickerson apparatus (in low-density solvent configuration). The extraction solvent was diethyl ether. In each batch 100 g of garlic cloves was blended with 20 ml of deionized water for 2 min. The sample flask was charged with the batch in 100 mL of deionized water. The micro-simultaneous steam distillation extraction procedure carried out for 2 h. For the condensation of steams the condenser was cooled with a solution of glacial water-glycol (−10 °C). The experimental procedure repeated with ethyl acetate. So, two garlic oil samples (s1 and s2) were collected.

2.3. Microwave-assisted hydrodistillation extraction (MWHD)

The microwave-assisted hydrodistillation setup performed in a modified LG 700 Watt max power

household microwave oven, using a steam distillation solvent extraction apparatus equipped with a 1 L sample flask, a pressure equalizing dropping funnel and a steam condenser. This apparatus used instead the classical Licke-Nickerson due to the vigorous foaming phenomenon during the procedure. The extraction solvent was diethyl ether (50 mL). In each batch 100 g of garlic cloves was blended with 20 mL of deionized water for 2 min. The sample flask was charged with the batch in 100 mL of deionized water and diethyl ether. The distillation time was 30 min for the isolation of the essential oil at maximum power. The organic extract was concentrated by a gentle nitrogen blowdown stream to 10 mL volume and a minor quantity of magnesium sulfate anhydrous was added. The experimental procedure was repeated with ethyl acetate. So, two garlic oil samples (s3 and s4) were collected.

2.4. Ultrasound-assisted extraction (USE)

Ultrasound-assisted extraction was performed in an ultrasound water bath (Sonorex, Super RK 255H type, 300 × 150 × 150 mm internal dimensions) by the mode of the indirect sonication, at the fixed-frequency 35 kHz. The temperature of the sonicated water bath was 25 °C. The sample flask was charged with the same quantity garlic as in the micro-steam distillation procedure. The solvent system extractant was, respectively, the same as above, diethyl ether 50 mL. Each garlic sample was sonicated three times for 30 min (three fractions per garlic sample). After the end of each son-

ication the solvent system extractant was introduced in a separation funnel and 20 mL of a saturated solution of NaCl was added. The funnel was well shaken and then left to rest. When the two layers were separated the organic layer was collected. The water layer was washed with another 20 mL of the extractant solvent. The whole organic extract was introduced again in the separation funnel and washed with 20 mL of a saturated solution of NaCl. The organic extract was concentrated by a gentle nitrogen blowdown stream to 10 mL volume and a minor quantity of magnesium sulfate anhydrous was added, in order to remove any traces of water. The experimental procedure repeated with ethyl acetate. So, two garlic oil samples (s5 and s6) were collected.

2.5. Gas chromatography (GC) analysis conditions

The garlic oil samples (s1–s6) were analyzed using a Hewlett Packard 5890 II GC equipped with a FID detector and HP-5ms capillary column (30 m × 0.25 mm, film thickness 0.25 µm). Injector and detector temperatures were set at 220 and 290 °C, respectively. Column temperature was raised from 50 to 220 °C with a rate of 3 °C/min, and held for 10 min. Helium was the carrier gas, at a flow rate of 1 mL/min. One microliter (1.0 µL) of the concentrate to 10 mL volume of each organic extract was injected manually and in the splitless mode. Quantitative data were obtained electronically from FID area percent data without the use of correction factors.

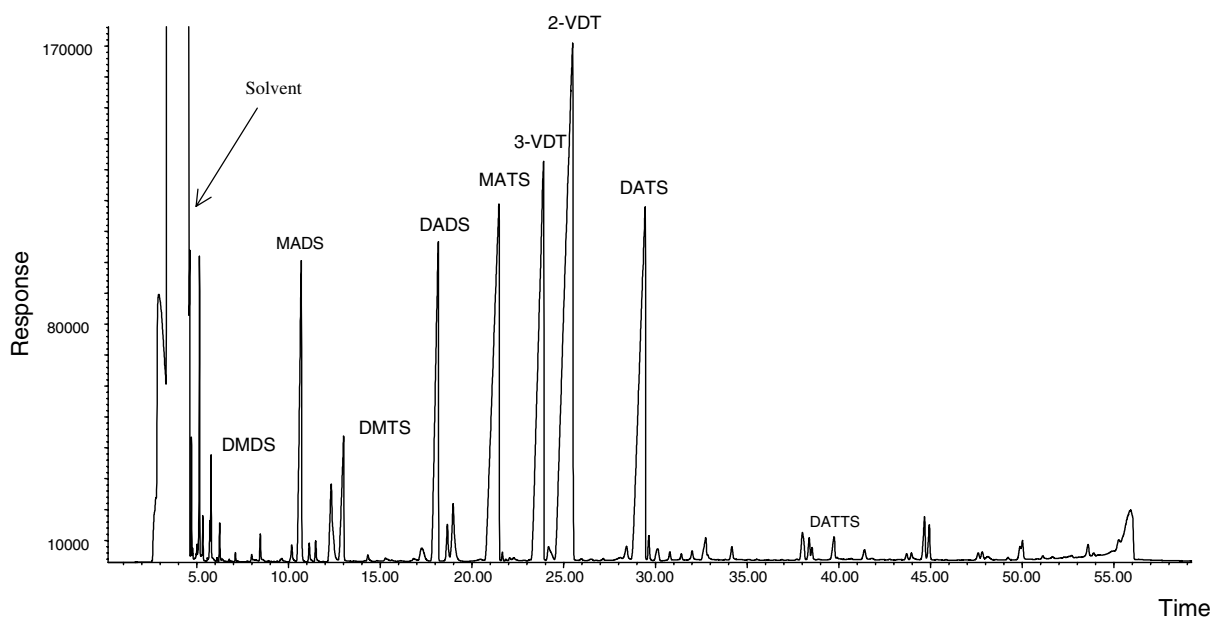


Fig. 2. Typical chromatograms of volatile fractions isolated from fresh garlic (*A. sativum*) cloves by microwave-assisted hydrodistillation extraction (MWH) in solvent ethyl acetate. The initial on the chromatograms indicates the compounds as below: dimethyl disulfide (DMDS), methyl allyl disulfide (MADS), dimethyl trisulfide (DMTS), diallyl disulfide (DADS), methyl allyl trisulfide (MATS), 3-vinyl-[4H]-1,2-dithiin (3-VDT), 2-vinyl-[4H]-1,3-dithiin (2-VDT), diallyl trisulfide (DATS), diallyl tetrasulfide (DATTS).

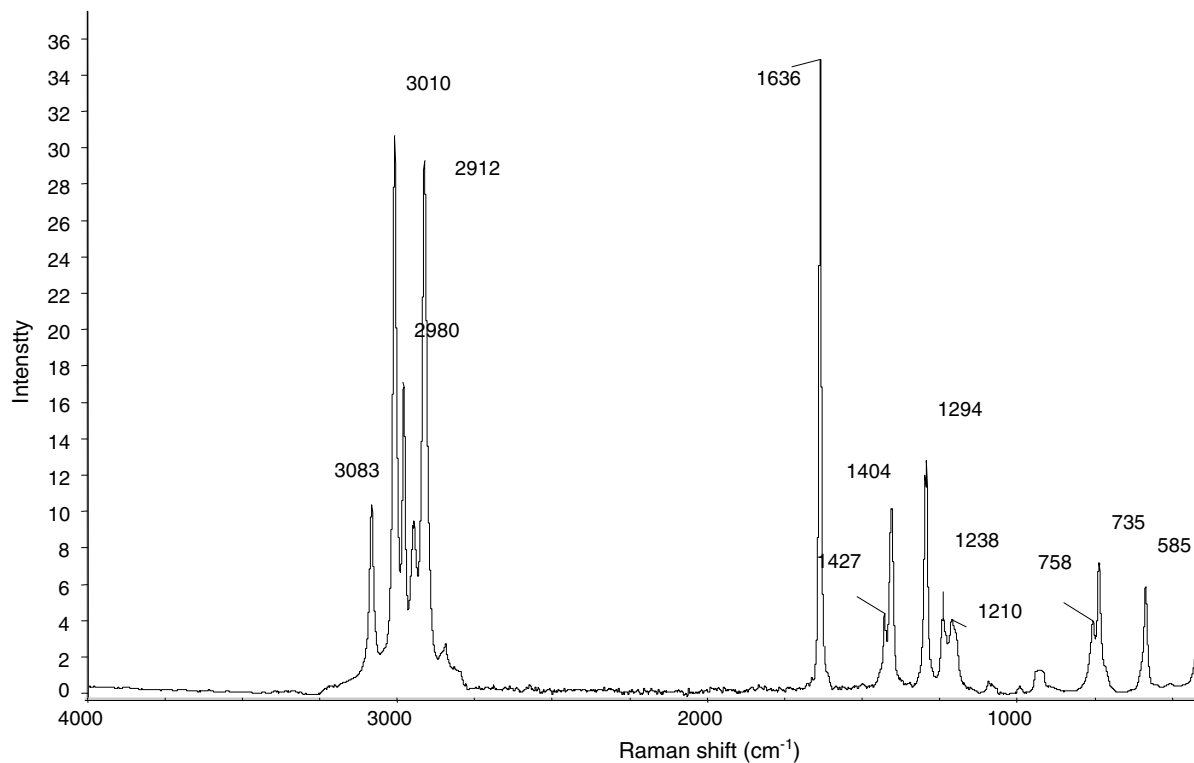


Fig. 3. FT-Raman spectrum of diallyl sulfide.

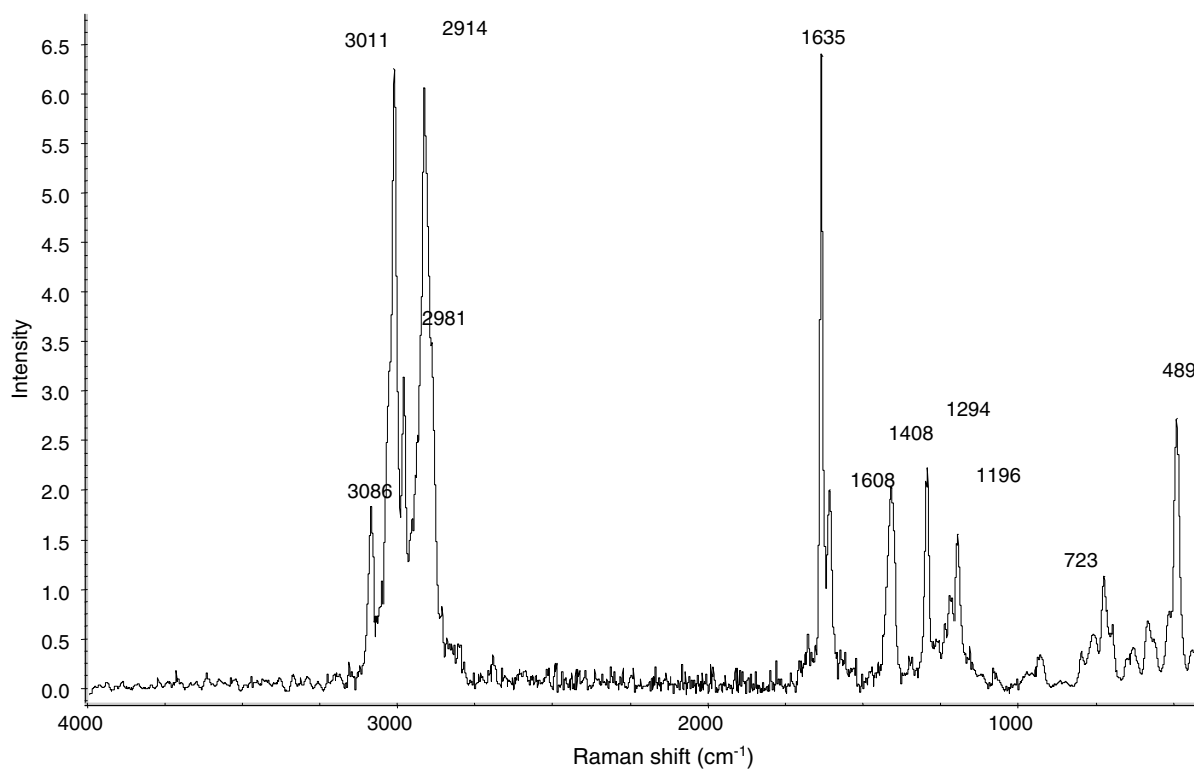


Fig. 4. FT-Raman spectrum of garlic oil extracted by ethyl acetate using microwave-assisted hydrodistillation extraction.

Table 2
Contents (% v/v) in acyclic components in standards correlated with A using FT-Raman method

Standard	Height at 1636 cm ⁻¹ (H)	Height at 1606 cm ⁻¹ (H ₁)	$H - H_0^a$	$H - H_0/H_1 = A$	$A \pm SD$	Volume (μL) of diallyl sulfide added to 100 μL of s5	Content in acyclic components (% v/v)
st1							
st1a	6.656	5.161	1.495	0.193	0.191 ± 0.006	4.6	16.7
st1b	6.671	5.156	1.515	0.196			
st1c	6.613	5.157	0.954	0.185			
st2							
st2a	11.425	5.041	5.766	1.144	1.143 ± 0.003	18.9	26.6
st2b	11.414	5.047	5.754	1.140			
st2c	11.434	5.039	5.774	1.146			
st3							
st3a	14.520	4.753	8.860	1.864	1.859 ± 0.004	39.7	37.5
st3b	14.478	4.751	8.818	1.856			
st3c	14.484	4.749	8.824	1.858			
st4							
st4a	15.616	4.150	9.956	2.399	2.406 ± 0.007	48.5	41.2
st4b	15.628	4.143	9.968	2.406			
st4c	15.660	4.146	10.000	2.412			
st5							
st5a	19.904	4.208	14.244	3.385	3.379 ± 0.006	66.9	47.7
st5b	19.848	4.205	14.188	3.374			
st5c	19.858	4.203	14.198	3.378			
st6							
st6a	21.668	4.206	16.008	3.806	3.808 ± 0.006	74.6	50.0
st6b	21.667	4.209	16.007	3.803			
st6c	21.690	4.203	16.030	3.814			
st7							
st7a	21.134	3.739	18.474	4.941	4.942 ± 0.005	91.9	54.5
st7b	24.139	3.743	18.479	4.937			
st7c	24.201	3.748	18.541	4.947			
st8							
st8a	28.590	3.670	22.930	6.248	6.242 ± 0.006	126.8	61.5
st8b	28.494	3.661	22.834	6.237			
st8c	28.536	3.666	22.876	6.240			
st9							
st9a	23.025	2.531	17.365	6.861	6.852 ± 0.008	162.2	66.7
st9b	22.976	2.529	17.316	6.847			
st9c	23.022	2.535	17.362	6.849			
st10							
st10a	17.219	1.438	11.559	8.038	8.038 ± 0.009	266.8	76.2
st10b	17.141	1.430	11.481	8.029			
st10c	17.215	1.436	11.555	8.047			

^a The height average of triplicate spectra of sample s5 at 1636 cm⁻¹ was 5.660.

2.6. Gas chromatography-mass spectrometry (GC-MS) analysis conditions

Analysis of garlic oil samples (s1–s6) was performed under the same conditions with GC, using a Hewlett Packard 5890 II GC, equipped with a HP 5972 Mass selective detector and a HP-5ms capillary column (30 m × 0.25 mm, film thickness 0.25 μm). For GC/MS detection an electron ionization system with ionization energy of 70 eV was used. Injector and MS transfer line temperatures were set at 220 and 290 °C, respectively.

Tentative identification of the compounds was based on the comparison of their mass spectra with those of NIST 98 and Wiley 275 library data of the GC-MS system.

2.7. FT-Raman spectroscopy

FT-Raman spectra of standards (st1–st10) and samples (s1–s6) were recorded in triplets using a Nicolet 750 FT-Raman spectrometer, equipped with a Nd:YAG laser source that emits at 1064 nm. In addition a CaF₂

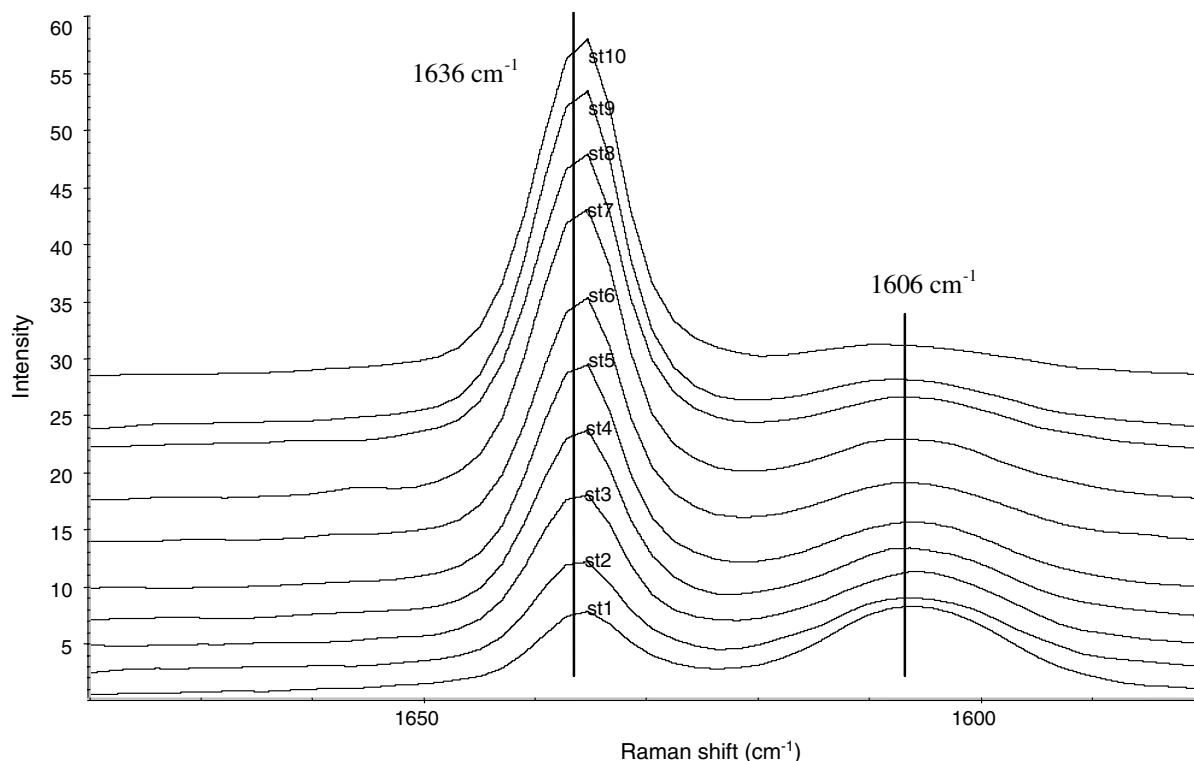


Fig. 5. Spectral region ($1750\text{--}1500\text{ cm}^{-1}$) of FT-Raman spectra of standards.

beam splitter, an indium–gallium–arsenide (InGaAs) detector and 180° backscattering geometry were used in the spectrometer. Routine procedures such as bench alignment and fine tuning of the spectrometer were held before each batch of measurements. Sample cells were Wimad WG-SM NMR tubes 4.97 mm outer diameter and 0.38 mm wall thickness. Optimal conditions (recording time, signal-to-noise ratio) were determined at 100 scans (3 min) with a resolution of 4 cm^{-1} . Each Raman spectrum was automatically smoothed and baseline corrected using the proper functions from the built-in spectrometer software Omnic 3.1. Then the peak heights at 1636 and 1606 cm^{-1} , from baseline, were measured and the height average of each one triplicate standard and sample was calculated.

3. Results and discussion

3.1. GC results

A typical total ion chromatogram (TIC) of the essential oil isolated from garlic (s5 sample) is shown in Fig. 2. The relative percentage contents of all components found in the garlic oil isolated with SDE, MWHD and USE with diethyl ether and ethyl acetate are shown in detail in Table 1. Compounds that could not be identified with the above GC libraries are referred to as unidentified and their total content is

shown separately. The sulfur compounds can be further classified into two major groups according to their cyclic or acyclic structure. The cyclic group contains the two vinyl dithiin isomers while the acyclic group consists of various mono to polysulfides (substituted by different R-). This group contains both saturated and unsaturated compounds but only the total relative percentage of the unsaturated compounds is considered.

Content of total unsaturated components fluctuated between 12.7% and 77.4%. The highest content was derived from the use of SDE with diethyl ether, while the lowest with USE and diethyl ether.

3.2. FT-Raman results

In Figs. 3 and 4 are presented the FT-Raman spectra of diallyl sulfide and typical garlic oil. In the region between 3100 and 2800 cm^{-1} four major peaks are present. The first at $3086\text{--}3083\text{ cm}^{-1}$ corresponds to asymmetric stretch vibration of $=\text{CH}_2$, the second at $3011\text{--}3010\text{ cm}^{-1}$ to C–H stretching, the third at $2981\text{--}2980\text{ cm}^{-1}$ to symmetric stretch vibration of $=\text{CH}_2$ and the fourth at $2914\text{--}2912\text{ cm}^{-1}$ to $-\text{CH}_2-$ stretching. The second region from 1700 to 1000 presents four peaks in diallyl sulfide and five in garlic oil. The very intense peak at $1636\text{--}1635\text{ cm}^{-1}$ is assigned to C=C stretching vibration of the allyl group. The peak at 1606 cm^{-1} , which is present only in the FT-Raman

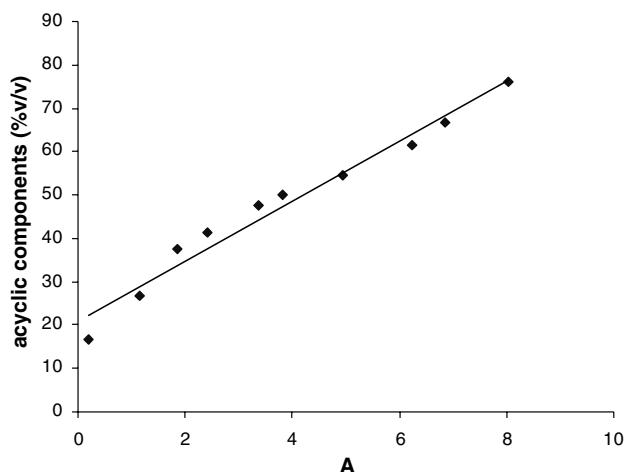


Fig. 6. The calibration curve of acyclic components.

spectrum of garlic oil, is assigned to the conjugated double bond of the dithiin ring. The double peak at $1428\text{--}1401\text{ cm}^{-1}$ may be assigned to stretching of $\text{--CH}_2\text{--}$ group while $\text{CH}_2=\text{CH--}$ stretching is shifted to 1294 cm^{-1} . The skeletal vibration of diallyl sulfide molecule causes the double peak at $1220\text{--}1198\text{ cm}^{-1}$. The last region from 1000 to 400 is very interesting as it contains C–S and S–S vibrations. The double peak at 755 and 723 cm^{-1} is due to symmetric stretch of C–S bond while the asymmetric vibration of the same group is present at 585 cm^{-1} . The peaks near 489 are due to the disulfide bridge stretching, shifted when more S atoms are present. The above peaks have been assigned

according to Baranska, Labudzinska, and Terpinski (1987) and Dean (1999).

The most interesting peaks are at 1636 and 1606 cm^{-1} which are correlated with the unsaturated acyclic and cyclic components, respectively. The garlic oil which was extracted by ethyl acetate using MWHD (sample s5) is the poorest sample in acyclic components (Table 1). When diallyl sulfide was added in $100\text{ }\mu\text{L}$ of the above sample (Table 2) the peak height at 1636 cm^{-1} was increasing (Fig. 5). Therefore, the difference of the peak height between the standard and sample s5 can be attributed to the amount of acyclic components added. Furthermore, the content (% v/v) in unsaturated acyclic components of garlic oils, whose content is higher in acyclic components than sample s5, can be correlated with the increase of the peak height at 1636 cm^{-1} . The height average of triplicate spectra of sample s5 at 1636 cm^{-1} was 5.660 . The above increase of the peak height at 1636 cm^{-1} was normalized using the peak height at 1606 cm^{-1} . Thus, the formula applied for this calculation is as follows:

$$\frac{\text{Height}_{1636} - 5.660}{\text{Height}_{1606}} = A.$$

Table 2 shows the ratios A of the 10 standards and the content (% v/v) in unsaturated acyclic components. There is a linear relationship between the ratios A of the 10 standards and the content (% v/v) in unsaturated acyclic components (Fig. 6). The empirical equation of calibration curve is:

Table 3
Contents (% v/v) of samples in acyclic components using FT-Raman method

Samples	Height at 1636 cm^{-1} (H)	Height at 1606 cm^{-1} (H_1)	$H - H_0^a$	$H - H_0/H_1 = A$	Content in acyclic components (% v/v) \pm SD
SDE-diethyl ether (s1)					
s1a	17.872	1.532	12.212	7.971	75.3 ± 0.5
s1b	17.756	1.537	12.096	7.870	
s1c	17.673	1.535	12.013	7.826	
SDE-ethyl acetate (s2)					
s2a	17.027	1.426	11.367	7.971	75.0 ± 0.8
s2b	16.694	1.423	11.034	7.754	
s2c	16.732	1.420	11.072	7.797	
MWHD-diethyl ether (s3)					
s3a	23.536	3.698	12.786	5.508	58.6 ± 0.9
s3b	25.419	3.705	19.759	5.333	
s3c	26.278	3.695	20.618	5.580	
MWHD-ethyl acetate (s4)					
s4a	22.669	4.207	17.009	4.043	48.4 ± 0.5
s4b	22.464	4.201	16.804	4.000	
s4c	22.110	4.204	16.450	3.913	
USE-ethyl acetate (s6)					
s6a	14.321	4.497	8.661	1.926	33.3 ± 0.8
s6b	13.410	4.493	7.750	1.725	
s6c	13.477	4.495	7.817	1.739	

^a The height average of triplicate spectra of sample s5 at 1636 cm^{-1} was 5.660 .

Content in unsaturated acyclic components (%v/v)

$$= (20.9 \pm 1.9) + (6.9 \pm 0.4) \times A \quad (r = 0.97, n = 3).$$

Detection limits are in the range 16.7–76.2% v/v and the % RSD fluctuated from 0.1% to 3.1%.

Contents (% v/v) of garlic oils in unsaturated acyclic components were measured by using the earlier empirical equation (Table 3). The % RSD fluctuated from 0.7% to 2.4%.

Content of total unsaturated components fluctuated between 33.3% and 75.3%. The highest content was derived from the use of SDE and diethyl ether (75.3%), while the lowest with USE and ethyl acetate (33.3%). In addition, the use of SDE and ethyl acetate, MWH and diethyl ether and finally MWH and ethyl acetate resulted 75.0%, 58.6% and 48.4%, respectively (Table 3).

In the case of s1, s2 and s3 samples the quantitative analysis results are considered similar, and the content of unidentified compounds is limited to 3–4.2%. On the contrary, samples s4 and s6 show considerable variation between the GC and the FT-Raman analysis. This may be the result of increased content in unidentified compounds of the garlic oil. It is ranging between 7.2% and 11.7% (Table 1), probably consisting mainly of unsaturated acyclic compounds.

In conclusion, the quantitative analysis of unsaturated acyclic components can be determined by FT-Raman spectroscopy. The analysis results come in agreement with the GC method results and the garlic oils were found to contain 33.3–75.3% of the components in question. The main advantage of this method over the existing GC method is its simplicity, immediacy, speed and being non-destructive to the sample. The perspectives of this technique are wide in the field of heat sensitive components analysis, where traditional methods fail to report accurate results. Garlic oil being a highly unstable molecular mixture demonstrates this property of the proposed method.

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