Extraction and characterization of essential oil of garlic (Allium sativa L.)

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Abstract

The essential oil was extracted from garlic powder by soxhlet extraction method using ethanol as a solvent. The yield of essential oil was influenced by extraction time and temperature. The maximum extraction yield was 16.55% under treatment- T7 (50 °C for 4 hours). The density and refractive index of the oil was 0.875 (g/ml) and 1.52 respectively. The oil is light yellowish in colour with a pungent odour. The maximum TOAC (12.01 mM α tocopherol per ml of essential oil) was found under T3 treatment. Essential oil was analyzed by gas chromatography–mass Spectrometry (GC-MS). The major chemical compound were: diallyl disulfide (48.42%), allyl methyl trisulfide (7.27%), trisulfide, di-2 propenyl (3.46%), and dailly sulfide (7.64%). The results revealed that T7 was the best treatment among all treatments. This indicates the feasibility of garlic oil production at a commercial scale for culinary and medical utilization.

Keywords: Garlic essential oil, chemical composition, refractive index, TOAC, Allium sativum

1. Introduction

Allium sativum L., commonly known as garlic, belongs to the onion family, liliaceae. Garlic was likely originated in Central Asia and it has been in use throughout the world for both culinary and medicinal purposes [1-2]. The garlic oil, rich in sulfured organic compounds, contains a variety of sulfides such as diallyl disulfide and dilyl trisulfide [3-7]. It is used not only as a flavoring agent, food preservative but also in the prevention and treatment of several illnesses [8, 9]. In the pharmaceutical industry, it is much used due to its anticarcinogenic, antithrombotic and antplatelet aggregation properties. The regular consumption of garlic oil can reduce blood pressure, prevent heart disease including atherosclerosis, high cholesterol and cancer [10]. Recent biological and pharmacological research [11-21] confirms these medicinal properties showing that garlic oil has an antibiotic, antioxidant, anti-viral, anti-fungal, antimicrobial, anticarcinogenic and immunomodulatory effect and garlic can be used to prevent nausea, diarrhea, ease coughs and even in treatment conditions such as malaria and cholera. It is an immune system enhancer [22]. Some studies have found lower rates of certain types of cancer in people who use it regularly.

Being the second largest producer of garlic, India maintains surplus quantity most of the times that remains unutilized. India provides 5.2% of the total world production followed by China with 80% share in the global market. The other major producers are Bangladesh and Egypt followed by Korea, Russia and others. In India Madhya Pradesh, Rajasthan, Gujarat, Orissa, Uttar Pradesh and Maharashtra are the main states where garlic is grown commercially with an average yield of 6-8 tonnes/ha. Madhya Pradesh is the leading garlic producing state with the production of 4.24 lakh tones accounting to about 26.25% of total Indian production and a yield of 7.86 tonnes/ha.

In India, due to lack of poor post-harvest handling practices, suitable storage, processing facilities, heavy losses are incurred both in terms of quality and quantity. This may be attributed to respiration, transpiration and microbiological spoilage. Though garlic is produced abundantly and consumed as such, little efforts have so far been made to produce garlic essential oil from garlic powder. Garlic is a semi perishable commodity and nearly 30% of the crop is wasted due to respiration and microbiological spoilage during storage [23], which needs to be addressed. Therefore, it is important to diversify its utility forms.
Extraction of essential oil is a major food processing operation in the food industry for utilization of this surplus garlic in terms of value addition and income generation and thereby minimize wastage. There are different methods for extraction of essential oil. In this study, the essential oil was extracted by soxhlet extraction method using ethanol as a solvent. This study had four objectives: 1) Optimization of a process parameter, 2) Determination of physical properties, 3) To find out chemical composition of the extracted oil, 4) Evaluation of antioxidant capacity.

2. Materials and Methods
Fresh garlic (local variety) was procured from Tech market, IIT Kharagpur and during the experiments, all the samples were stored in our lab at appropriate conditions (dark, 27 °C). The garlic cloves were cut into two equal-size manually by stainless steel knife with the utmost care and immediately kept into the oven at 60°C to dry for 48 hours and the powder was made by mechanical tools.

2.1. Extraction of Essential Oil
The solvent extraction method was conducted with a soxhlet extractor using commercial ethanol at different temperature of 50, 60 and 70°C for 2, 3 and 4 hours. The combination of temperature and time were determined in a preliminary set of experiments. Three replicates were carried out for all nine treatments to reduce the error. Garlic powder was used (10g) at 1:20, sample to solvent ratio. The oil was obtained after the solvent was evaporated by placing over a water bath (LABARD, LI-WBPR-14A) for about 2-3 hours under reduced temperature (50°C) and refluxing at 70°C to remove any excess solvent [2]. The extracted garlic oil was stored in a refrigerator at 4°C for subsequent physico-chemical analyses.

2.2. Antioxidant Activity
The total antioxidant capacity (TAOC) of the essential oil samples was measured as previously described [24]. Briefly, 40µl of essential oil was mixed with a reagent solution (0.6 N sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) in an Eppendorf tube in the ratio of 1:100 (v/v) and the tubes were capped and incubated at 95°C for 90 minutes. The addition of essential oil to the reagent solution caused discoloration after incubation. This indicates the scavenging capacity of essential oil. Samples were cooled at room temperature and absorbance was measured at 695 nm using a spectrophotometer (Epoch 2, BioTek, U.S.A.). All determinations were performed in duplicate. TAOC of the samples were expressed as equivalents of mM-α-tocopherol per ml of essential oil and was calculated as follows:

\[ A = \alpha \times L \]

Where
A= Absorbance
\( \alpha \)= Extinction co-efficient (4×103 M-1 cm-1)
C= Concentration (molar)
L= Path length (1 cm)

\[ TOAC = \frac{A}{(\alpha \times L) \times amount \ of \ sample} \] mM/α tocopherol/ml

2.3. Chemical Composition
The volatile oil extracted from garlic powder was subjected to GC-MS analysis as previously described [25-32]. A GC-MS (Thermo Scientific, Trace 1300), GC (TRACE-GC ULTRA) and MS (POLARISQ) instrument was used to study the composition of extracted essential oil. This instrument was operated in the electron impact (EI) mode set at electron energy 70eV and a scan range of 0.00 amu–100 amu, with a scan rate of 3.0 scans per second. DB-5MS column of 30 m length with 0.25 mm inner diameter was used. Helium gas (99.99%) was used as a carrier at a constant flow rate of 1 ml min-1 on the column head. The temperature of the injector was set at 250 °C and the temperature of the ion source was set at 230 °C. The temperature of the GC oven was programmed to be 50 °C initially and was programmed to increase at a rate of 5 °C/min to a final temperature of 260 °C. The sample was prepared by diluting the essential oil in a ratio of 1:10 with methanol and 1.0 µl volume of sample was injected into the instrument with a split ratio of 30:1. The obtained mass spectra were thoroughly screened and individual components of essential oils were quantified by relative peak percent area. Identification of each quantified components was done by comparing their mass fragmentation pattern with components stored in the spectrometer database using NIST mass spectral library (Version 2014).

2.4 Removal of Milky Emulsion and Excess Solvent
Since the milled substrate showed a tendency to agglomerate during extraction, optimal particle size was determined in a preliminary set of experiments as the smallest that did not cause perceptible agglomeration problems and this size was 1-3 mm. Some essential oil extracted with milky emulsion (Fig. 1) was centrifuged at 3000 rpm for 5 minutes by a high-speed refrigerated research centrifuge –RC 4100 F. By this, the milky emulsion stuck around the surface of the bottle and clean essential oil including solvent was separated by pipet, then placed in a water bath for removing excess solvent.

3. Results and Discussion
3.1. Yield of Garlic Oil
The yield of garlic essential oil changed with the process temperature, and time (Table 1). The best treatment for this research work was T7 (50°C for 4 hour). The maximum extraction yield was 16.55% (db) among the nine treatments. By optimizing the process parameter, the best combination of the parameters were found to be 4 hours and 50°C. These parameters can be ideal one to obtain the maximum yield at a commercial level. Thus, in the present study much higher oil yield was obtained compare to that reported by Ali Rafe et al. 2014 [33]. They found that maximum yield were 5.5, 6 and 7% for steam distillation, solvent method and SCF-CO₂, respectively.

Fig 1: Essential oil + Solvent+ Emulsion

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Table 1: Extraction yield %

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Time (h)</th>
<th>Temperature (°C)</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>2</td>
<td>50</td>
<td>7.55</td>
</tr>
<tr>
<td>T2</td>
<td>2</td>
<td>60</td>
<td>9.33</td>
</tr>
<tr>
<td>T3</td>
<td>2</td>
<td>70</td>
<td>11.88</td>
</tr>
<tr>
<td>T4</td>
<td>3</td>
<td>50</td>
<td>14.55</td>
</tr>
<tr>
<td>T5</td>
<td>3</td>
<td>60</td>
<td>13.55</td>
</tr>
<tr>
<td>T6</td>
<td>3</td>
<td>70</td>
<td>13.75</td>
</tr>
<tr>
<td>T7*</td>
<td>4</td>
<td>50</td>
<td>16.55</td>
</tr>
<tr>
<td>T8</td>
<td>4</td>
<td>60</td>
<td>15.88</td>
</tr>
<tr>
<td>T9</td>
<td>4</td>
<td>70</td>
<td>15.33</td>
</tr>
</tbody>
</table>

3.2. Physical properties
The Physical examination of the extracted oil was conducted and presented in Table 2. The properties were moisture content, density, refractive index, appearance and odour. The moisture content of the peeled garlic cloves was (63.14±1%). The density and refractive index were 0.875±1 (g/ml) and 1.52 at room temperature which falls within the range of volatile oil in general. The appearance of extracted oil was light yellowish and it had pungent odour.

Table 2: Physical properties of extracted garlic essential oil

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture content (%)</td>
<td>63.14±0.38</td>
</tr>
<tr>
<td>Density (g/cm3)</td>
<td>0.873±0.003</td>
</tr>
<tr>
<td>Refractive index</td>
<td>1.52±0.02</td>
</tr>
<tr>
<td>Appearance</td>
<td>Light yellow</td>
</tr>
<tr>
<td>Odour</td>
<td>Pungent</td>
</tr>
<tr>
<td>Oil yield (%)</td>
<td>16.55±0.33</td>
</tr>
</tbody>
</table>

3.3. Chemical Composition of Extracted Oil
The essential oil compositions were determined by GC-MS. The qualitative and quantitative differences of compounds are presented in Table 3. The total ion chromatogram of garlic essential oil is shown in Fig. 3. The major compounds in the extracted oil were diallyl disulfide (48.42%), allyl methyl trisulfide (7.27%), trisulfide, di-2 propenyl (3.46%) and daillyl sulfide (7.64%). There was some loss of volatile compounds of essential oil, may be during oven drying of garlic slices. Indeed these chemical compounds covered more than 85% of GC profile. Sulfide compound was dominated among all compounds. This result is slightly different from other authors (Douiri et al. 2013) [34] who reported that garlic essential oil obtained by Clevenger hydrodistillation contained diallyl disulfide (16.0%) and allyl methyl trisulfide (10.9%). Similarly, Rao et al. 2007 [35] have analyzed six geographical varieties of essential oils extracted by steam distillation from fresh garlic grown in India and found that diallyl disulfide (27.1–46.8%) and diallyl trisulfide (19.9–34.1%) dominated in the oil followed by allyl methyl trisulfide (8.3–18.2%) and allyl methyl disulfide (4.4–12.0%). It can be expected that this oil may be commercialized for medicinal purposes in view of its reported prophylactic and curative profile.

Table 3: Results of GC-MS analyses of extracted oil

<table>
<thead>
<tr>
<th>RT (min)</th>
<th>Compounds</th>
<th>Composition %</th>
</tr>
</thead>
<tbody>
<tr>
<td>34.91</td>
<td>Diallyl disulfide.</td>
<td>48.42%</td>
</tr>
<tr>
<td>3.28</td>
<td>Dality sulfide</td>
<td>7.64%</td>
</tr>
<tr>
<td>22.44</td>
<td>Ally methyl trisulfide.</td>
<td>7.27%</td>
</tr>
<tr>
<td>17.25</td>
<td>Trisulfide, di-2 propenyl</td>
<td>3.46%</td>
</tr>
<tr>
<td>55.94</td>
<td>Hydrazine, methyl</td>
<td>5.75%</td>
</tr>
<tr>
<td>56.24</td>
<td>2-Propanone, 1-hydroxy</td>
<td>5.81%</td>
</tr>
<tr>
<td>51.10</td>
<td>1,2-Cyclopentanediene</td>
<td>1.24%</td>
</tr>
<tr>
<td>53.43</td>
<td>Cyclopropane carboxylic acid 1-amino</td>
<td>0.52%</td>
</tr>
<tr>
<td>52.95</td>
<td>Benzoic acid, 2-methyl</td>
<td>3.22%</td>
</tr>
<tr>
<td>58.08</td>
<td>3-Vinyl-1, 2-dithiacyclohex-5-en</td>
<td>1.13%</td>
</tr>
<tr>
<td>54.17</td>
<td>1H-Pyrrole, 1-methyl</td>
<td>0.75%</td>
</tr>
</tbody>
</table>

3.4. Antioxidant capacity
The total antioxidant capacity of essential oil was analyzed for each treatment and presented in Fig. 2. The maximum total antioxidant capacity was 12.018 mM α tocopherol per ml of essential oil for T3 treatment (70 °C for 2 hours). The results indicate that for a specific duration of time with every 10 °C rises in temperature there was an increase in antioxidant activity. It may be due to phenolic compound and the sulfur compound was more active at 70 °C temperature as compare to 50 °C and 60 °C temperature or these compound may be extracted more at 70 °C.
4. Conclusions
This study has demonstrated that there is a significant impact of temperature and time on the yield of essential oil. The best parameters are 4 hours and 50°C for obtaining the maximum yield. These parameters can be considered as an ideal one for commercial production. The physical properties i.e. moisture content, density, refractive index, appearance and odour were within the range of essential oil. The refractive index of extracted oil can be used for its identification from other edible oil sources. These properties indicates the feasibility of garlic oil production for commercial purposes. The extracted oil was found to be a very good antioxidant and it can not only be used for food preservation but also for prophylactic and therapeutic uses. The principal chemical compounds detected were: diallyl disulfide (48.42%), allyl methyl trisulfide (7.27%), trisulfide, di-2 propenyl (3.46%), and diallyl sulfide (7.64%). Sulfide compound dominated among the all compounds. It can be expected that this oil can be commercialized for medicinal and culinary purposes.

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6. COI Statement: The authors declared that they have no conflict of interest

7. References


