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DERIVATIZATION AND QUANTITATIVE ANALYSIS OF S-ALLYL-CYSTEINE IN CALLUS OF GARLIC (*Allium sativum* L.) VIA HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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Abstract

S-allyl-cysteine is a non-volatile organosulfur compound commonly present in garlic (*Allium sativum* L.) with promising medicinal properties such as cholesterol-lowering, hepatoprotective and neuroprotective effects as well as antidiabetic, anticancer, antioxidant and anti-inflammatory activities. In an attempt to monitor the production of this compound in garlic callus, a protocol was developed for its quantitative analysis. Standard S-allyl-cysteine was derivatized with dansyl chloride to increase the sensitivity and stability of its amino group prior to detection via High-Performance Liquid Chromatography (HPLC). Molar ratios of S-allyl-cysteine standard to dansyl chloride used at 1:1, 1:5, 1:10, 1:20, 1:30 and 1:40 resulted in 0.1796 ± 0.0014 µg, 0.3173 ± 0.0005 µg, 0.5872 ± 0.0011 µg, 0.8110 ± 0.0005 µg, 0.8172 ± 0.0004 µg and 0.8190 ± 0.0003 µg of derivatized S-allyl-cysteine, respectively. Based on the previous report, the fresh garlic contained S-allyl-cysteine at approximately 20 µg/g, thus molar ratios of 1:20, 1:40, 1:60, 1:80, and 1:100 of S-allyl-cysteine were subsequently employed to optimize the derivatization step. The S-allyl-cysteine contents in the fresh garlic determined with derivatization were at 0.1286 ± 0.0002 µg/g, 0.1289 ± 0.0004 µg/g, 0.1299 ± 0.0003 µg/g, 0.1299 ± 0.0002 µg/g and 0.1299 ± 0.0005 µg/g, respectively. The S-allyl cysteine production was also investigated in garlic callus cultured on Murashige and Skoog solid medium with 2,4-dichloropheoxyacetic acid (2,4-D) at a concentration of 0.05 mg/L for 8 weeks. The S-allyl-cysteine was extracted from the callus with methanol and derivatized with dansyl chloride following those molar ratios performed on the fresh garlic revealing 0.2331 ± 0.0008 µg/g, 0.2238 ± 0.0005 µg/g, 0.1990 ± 0.0004 µg/g, 0.1941 ± 0.0005 µg/g and 0.1823 ± 0.0002 µg/g, respectively. In the case of biological samples like those from fresh garlic and garlic callus, the molar ratio of 1:20 of S-allyl-cysteine to dansyl chloride is proposed for feasible detection and quantitative analysis of S-allyl-cysteine. Moreover, with the conditions preliminarily used in this study, garlic callus was shown to have S-allyl-cysteine content of nearly double that found in the fresh garlic counterpart, suggesting that tissue culture is an alternative approach for S-allyl-cysteine biosynthesis.

INTRODUCTION

Garlic (*Allium sativum* L.) is a bulbous perennial plant of the family Liliaceae, which can grow up to 1.2 m in height and is recognized by its distinctive aroma and pungent taste [1]. Various garlic culinary preparations have been developed, including garlic oil, macerated garlic oil, and garlic powder, which are simply used as food ingredients. Garlic has also been employed in both traditional and modern medicine worldwide. In addition to common nutrients like carbohydrates, proteins and lipids, garlic is a rich source of minerals [2]. Garlic is a good source of essential oil. The essential oil compositions of garlic obtained by different distillation methods have been investigated [3]. The essential oil from garlic has also been extracted and evaluated for production at a commercial scale for culinary and medical applications [4]. Organosulfur compounds (OSCs) in garlic have been extensively studied and concluded to responsible for the distinctive flavour and aroma. Major organosulfur compounds providing these beneficial effects are the allicin and ajoene, among other volatile and non-volatile sulfur-containing compounds. Researchers are interested in the potential applications of organosulfur compounds derived from garlic to prevent and treat chronic diseases, such as cancer, cardiovascular and age-related diseases [5].

OSCs are present in various amounts in different breeds of garlic. The content of organosulfur compounds in garlic bulbs changes during cultivation and storage. Some volatile compounds are converted to non-volatile compounds for stable accumulation [6]. Diallyl sulfides and mono sulfides are volatile and majorly provide garlic with its taste and odor. However, several less odorous sulfur compounds discovered in aqueous extracts of garlic like S-allyl-cysteine (SAC) or S-allyl-mercaptocysteine (SAMC) are found in plants of the *Allium* genus have also attracted much attention as bioactive compounds [7].

S-allyl-cysteine is a sulfur-containing amino acid in garlic; cysteine is conjugated with an allyl group at the sulfur atom to form this compound [8]. This water-soluble compound is found in fresh garlic in low amounts and more abundant in aged garlic. The S-allyl-cysteine is biosynthesized by γ-Glutamyltransferase, which converts γ-glutamyl-S-allyl-cysteine to S-allyl-cysteine (Figure 1) [9]. The S-allyl-cysteine is more stable in acid than in alkaline conditions because of the possible C-S bond cleavage at high pHs. The S-allyl-cysteine has been shown to possess of various biological benefits, including cholesterol-lowering, antidiabetic, anticancer and antihepatotoxic properties [10]. The production of S-allyl-cysteine from garlic tissue culture in our laboratory was inspired by the report of high levels of S-allyl-cysteine in garlic after 2 years of storage. The main aim was to elicit garlic callus to produce S-allyl-cysteine at approximately the same or more significant amounts but in a shorter time period than that occurs typically in garlic.

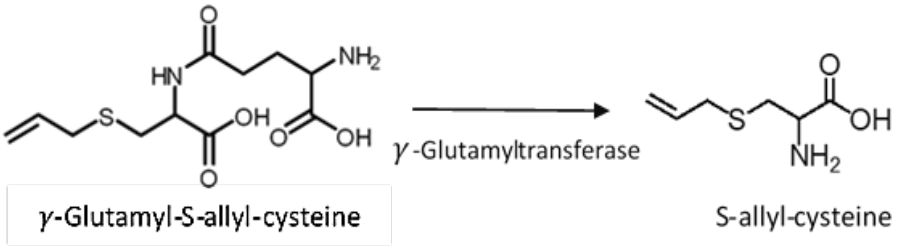

Figure 1. S-allyl-cysteine biosynthesis in garlic

Early in this study, the S-allyl-cysteine was previously detected in raw garlic and callus using thin-layer chromatography (TLC). However, when analyzed with HPLC, S-allyl-cysteine did not exhibit satisfactory resolution at either the visible or ultraviolet wavelengths. This may be due to the nature of its structure. Cysteine contains a carboxyl group that can absorb light in the 200-210 nm range and can be used for measurements but not widely used due to low sensitivity and must, therefore, be derivatized to improve detection sensitivity. Pre-column derivatization methods enable a sensitive HPLC analysis of amino acids (Figure 2). Dansyl chloride derivatization was originally applied to the sequential analysis of peptides and proteins. The amino acid analysis by dansylation was also documented [11]. It is commonly expected that the HPLC system uses a UV-Vis detector. However, not all analytes have strong UV absorption due to the absence of chromophore or fluorophore groups. Derivatization allows the addition of the moieties responsible for the absorption and the detection and quantification of analysis [12]. The 5-(Dimethyl amino) naphthalene-1-sulfonyl known as dansyl is a fluorophore that can be used as labeling reagent for derivatization of amine groups including aliphatic, biogenic and aromatic amines prior to their determination by HPLC [13]. The dansyl chloride (Dns-Cl) has several advantages over other derivatization methods, including a simple derivatization procedure, an increase in stability and sensitivity, a complete HPLC separation, and a higher detection limit stability strength more elevated [14].

In this study molar ratio of S-allyl-cysteine to dansyl chloride was optimized to assist in quantitative analysis of S-allyl-cysteine content in garlic samples.

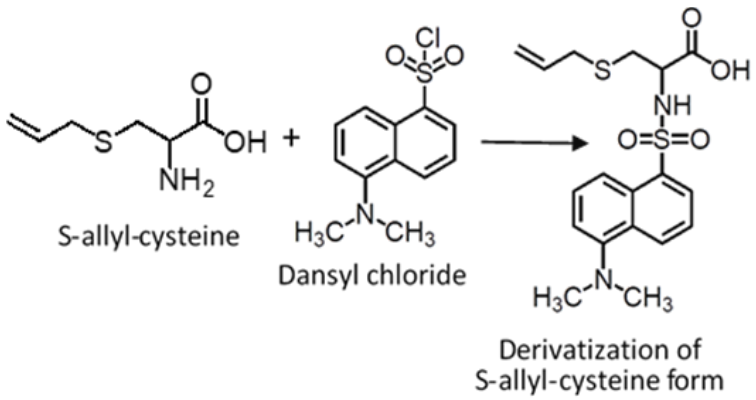

Figure 2. S-allyl-cysteine derivatization with dansyl chloride

MATERIALS AND METHODS

Chemicals and Plant Materials

The S-allyl-cysteine and dansyl chloride were purchased from Sigma Aldrich Chemical Co. Sodium acetate, boric acid and di-sodium tetraborate were obtained from LOBA Chemie. Methanol (MeOH) and acetonitrile (ACN) were from RCI Labscan. All chemicals were HPLC grade. The garlic (*Allium sativum* L.) bulbs for tissue culture were purchased from a local supplier in Chiang Mai, Thailand.

Solution Preparation

The S-allyl-cysteine (MW 161.22 g/mol) standard stock solution (25 mM) was prepared and diluted with deionized (DI) water to create the calibration curve with concentrations of 31, 62, 124, 186 and 250 µM. The range of concentrations chosen for standard curve construction was in response to the previous reports on the content of S-allyl-cysteine present in fresh garlic samples (20 µg/g) to cover those that might be detected in plant tissue culture samples. The SAC standard solution was prepared fresh prior to use. Dansyl chloride (MW 269.75 g/mol) was dissolved in acetonitrile to obtain 10 mM stock solution. Borate buffer at 20 mM was prepared from solutions of boric acid and di-sodium tetraborate in DI water. The pH was adjusted to 9.2 with sodium hydroxide (NaOH).

Callus Induction from Garlic Cloves

Garlic cloves were surface sterilized with 0.1% mercuric chloride, 70% ethanol and rinsed with sterile distilled water 3 times. Garlic explants were placed in Murashige and Skoog (MS) medium supplemented with 2,4-D at 1.5 mg/L and kinetin at 5.0 mg/L for callus induction. The culture was incubated under white fluorescent light for 16 hours at 2000-2500 lux at a temperature of 20 ± 2 ℃ in an incubator chamber for 4 weeks. Garlic callus was subcultured using the same medium in the presence of 2,4-D at a concentration of 0.05 mg/L. Garlic calli remained in the chamber and contents of S-allyl-cysteine were analyzed via HPLC at the end of 8 weeks.

Derivatization and Sample Preparation for Analysis

The S-allyl-cysteine standard at a concentration of 250 µM was derivatized with dansyl chloride at molar ratios of 1:0, 1:1, 1:5, 1:10, 1:20, 1:30 and 1:40, respectively to determine the best derivatization ratio for possible detection and quantitative analysis of S-allyl-cysteine. Borate buffer (20 mM, pH 9.2) was added to give a final volume of 1 mL. Various concentrations of S-allyl-cysteine (31-250 µM) derivatized at 1:20 molar ratio were used to construct the standard curve.

Fresh garlic and garlic callus were ground with a mortar and pestle in the presence of methanol at a weight per volume ratio of 1:5 and left at room temperature of 25 ± 2 ℃ for 2 days. The samples were centrifuged at 9,000 xg at 4 ℃ for 15 min. The extracts were mixed with dansyl chloride at molar ratios of 1:20, 1:40, 1:60, 1:80 and 1:100 according to the finding that fresh garlic contained S-allyl-cysteine at approximately 20 µg/g [15]. The reaction mixture was briefly shaken, then allowed to stand at room temperature for 15 min before filtering through a 0.45 µm syringe filter and analyzed using HPLC.

HPLC Analysis

Quantitative analysis of derivatized S-allyl-cysteine was analyzed using LiChrospher C-18 column (Lot. L88317) from Merck, Germany operated by HPLC system from Agilent, USA. The mobile phase consisted of a gradient system of methanol at 45% (0 min), 65% (5 min), 75% (20 min), and 45% (35 min) mixture with 50 mM sodium acetate pH 5.0 (adjusted by formic acid) to make up to 100% at each time frame, with a flow rate of 1.2 mL/min at room temperature of 25 ± 2 ℃ and detection wavelength of 250 nm. The injection volume was 30 µL. The analysis for derivatized S-allyl-cysteine and that from fresh garlic and callus garlic was conducted by HPLC in triplicate from 3 separate experiments.

Statistical Analysis

The mean and standard deviation of the means were calculated. The statistical analysis for the data obtained from this study employed Analysis of Variance (ANOVA) from Statistics software version 8. The multiple range significantly different LSD Tests were performed at $p < 0.05$. The results were given the mean of three independent experiments ± standard deviation.

RESULTS AND DISCUSSION

Derivatization of S-allyl-cysteine Standard with Dansyl Chloride

The derivatization step is recommended to help eliminate interferences from background and by-products. Furthermore, derivatization is frequently required to achieve the requisite sensitivity. However, detection and quantitative analyses of S-allyl-cysteine without derivatization were challenging due to undetectable signal and incomplete separation. Representative HPLC chromatogram suggested that underivatized S-allyl-cysteine was not detectable using these HPLC conditions (Figure 3). Compared to the underivatized from, the Dns-Cl derivatized one provided a clearer chromatographic profile with fewer interfering peaks. Derivatization reagents such as o-phthaldialdehyde (OPA), fluorenyl methyl chloroformate (FMOC), and dansyl chloride (Dns-Cl) have also been used for amine detection in conjunction with HPLC separation used in S-substituted cysteine derivative analysis. It was suggested that dansylation for quantitative purposes improved detection accuracy [15]. The DNS-Cl method is also appropriate for routine automated HPLC analysis of multiple samples due to its simplicity and excellent derivative stability. The DNS-Cl was found to be stable upon storage in the refrigerator at 4–5°C for at least 2 weeks [16].

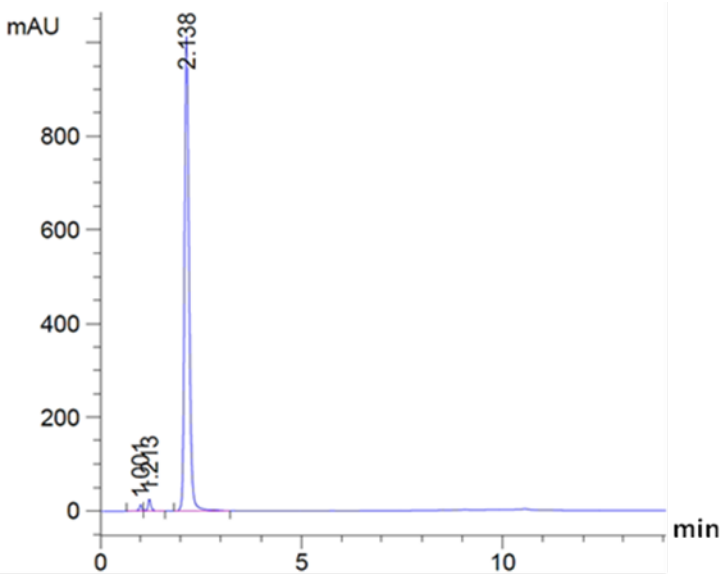

Figure 3. Representative chromatogram of S-allyl-cysteine standard without derivatization showing only solvent peak at a retention time of 2.138 min

The S-allyl-cysteine derivatized with dansyl chloride would provide stability and sensitivity of amino group in its structure. The quantitative analysis of derivatized S-allyl-cysteine standard was carried out using HPLC resulting in a sharp peak with a retention time of 8.191 ± 0.026 min at the molar ratio of 1:10 (Figure 4A), the retention time of 8.210 ± 0.038 min at the molar ratio of 1:20 (Figure 4B) and retention time of 8.267 ± 0.006 min at the molar ratio of 1:30 (Figure 4C). Table 1 shows the molar ratios of S-allyl-cysteine to dansyl chloride at 1:1 (0.1796 ± 0.0014 µg), 1:5 (0.3173 ± 0.0005), and 1:10 (0.5872 ± 0.0011 µg), with lower derivatized S-allyl-cysteine contents than those of 1:20 (0.8110 ± 0.0005 µg), 1:30 (0.8172 ± 0.0004 µg), and 1:40 (0.8190 ± 0.0003 µg). The molar ratios less than 1:20 did not provide sufficient dansyl chloride for complete reaction with S-allyl-cysteine. When the molar ratio of S-allyl-cysteine to dansyl chloride was at 1:20 and above, there was not much difference in the amounts of derivatized S-allyl-cysteine obtained considering microgram level. However, when those data were analyzed statistically, molar ratios of 1:20, 1:30 and 1:40 provided S-allyl-cysteine with significantly different amounts reported by LSD test ($p < 0.05$) (Table 1).

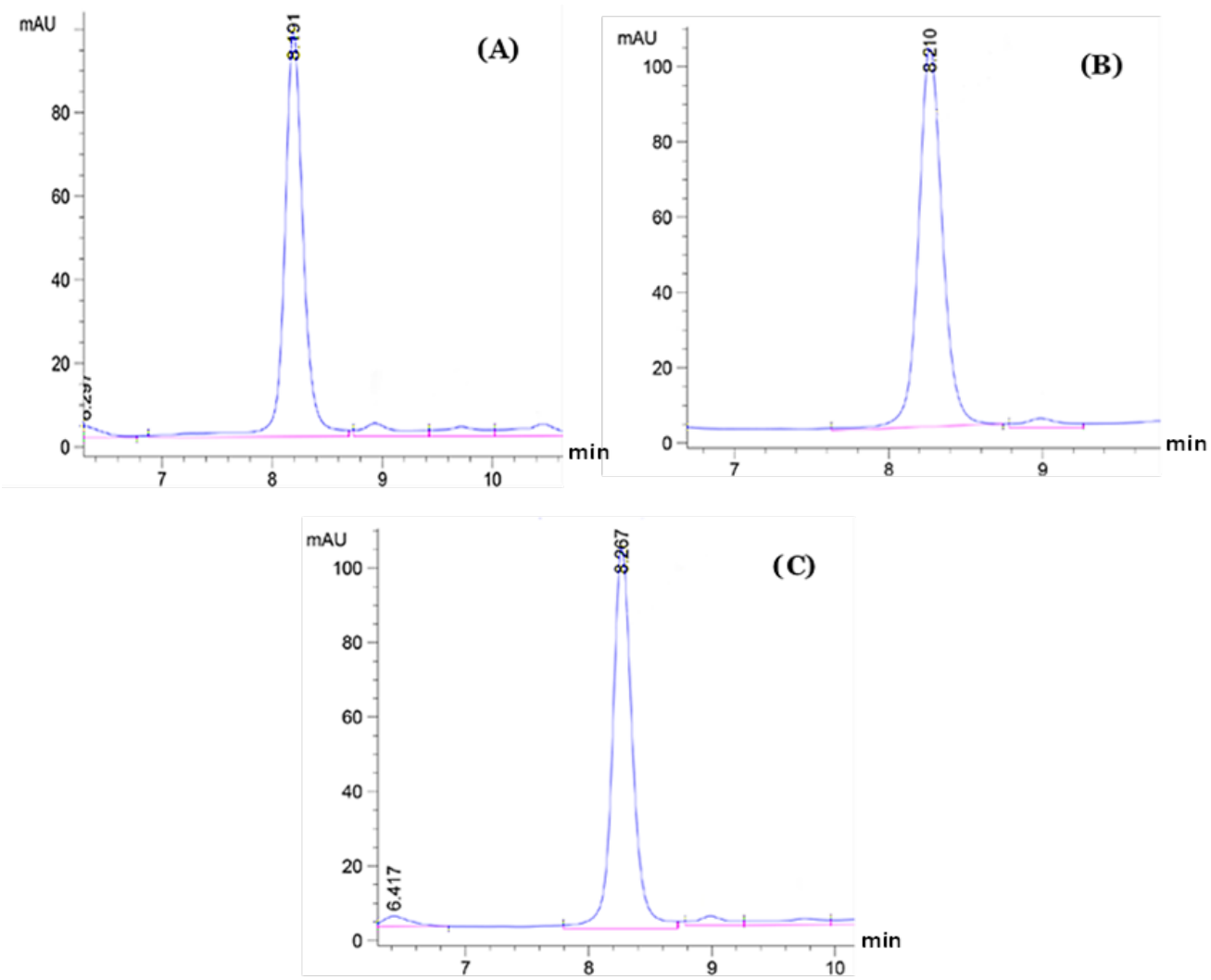

Figure 4. Representative chromatograms of S-allyl-cysteine standard derivatized with dansyl chloride at the molar ratios of (A) 1:10, (B) 1:20 and (C) 1:30 showing retention times of 8.191 ± 0.026, 8.210 ± 0.038 and 8.267 ± 0.006 min, respectively

Table 1. The molar ratio of S-allyl-cysteine standard to dansyl chloride with derivatized S-allyl-cysteine content and amount of dansyl chloride leftover

Molar ratio of S-allyl-cysteine standard to dansyl chloride	Derivatized S-allyl-cysteine content (µg)	Amount of dansyl chloride added to the reaction (µg)	Amount of dansyl chloride leftover from the reaction (µg)	Percentage of dansyl chloride leftover
1:0	0.0000 ± 0.0000 ^{g}	0.000	0.0000 ± 0.0000 ^{f}	0.00
1:1	0.1796 ± 0.0014 ^{f}	0.067	0.0000 ± 0.0000 ^{f}	0.00
1:5	0.3173 ± 0.0005 ^{e}	0.337	0.0315 ± 0.0024 ^{e}	9.35
1:10	0.5872 ± 0.0011 ^{d}	0.674	0.4796 ± 0.0052 ^{d}	71.15
1:20	0.8110 ± 0.0005 ^{c}	1.348	0.9453 ± 0.0003 ^{c}	70.13
1:30	0.8172 ± 0.0004 ^{b}	2.023	1.5845 ± 0.0031 ^{b}	78.32
1:40	0.8190 ± 0.0003 ^{a}	2.697	2.1180 ± 0.0017 ^{a}	78.52

All data in Table 1 are expressed as the mean ± standard deviation from triplicate determination. Different letters are multiple ranges significantly different reported by LSD test ($p < 0.05$)

Quantitative analysis of dansyl chloride leftover contents was carried out using HPLC. The representative chromatograms of dansyl chloride leftover from the reaction is shown in Figure 5 (A-C) demonstrating in a sharp peak with a retention time of 17.110 ± 0.009 min for the molar ratio of 1:20 (Figure 5A), the retention time of 17.463 ± 0.009 min for the molar ratio of 1:30 (Figure 5B) and retention time of 17.486 ± 0.004 min for the molar ratio of 1:40 (Figure 5C).

It was found that the amount of dansyl chloride leftover at molar ratio of 1:20 (0.9453 ± 0.0003 µg) was much less than those at 1:30 (1.5845 ± 0.0031 µg) and 1:40 (2.1180 ± 0.0017 µg) while S-allyl-cysteine contents (Table 1) at the molar ratio 1:20 and above were nearly the same. Therefore, the molar ratio of 1:20 was considered as an optimal molar ratio for dansylation of S-allyl-cysteine with the least amount of dansyl chloride leftover.

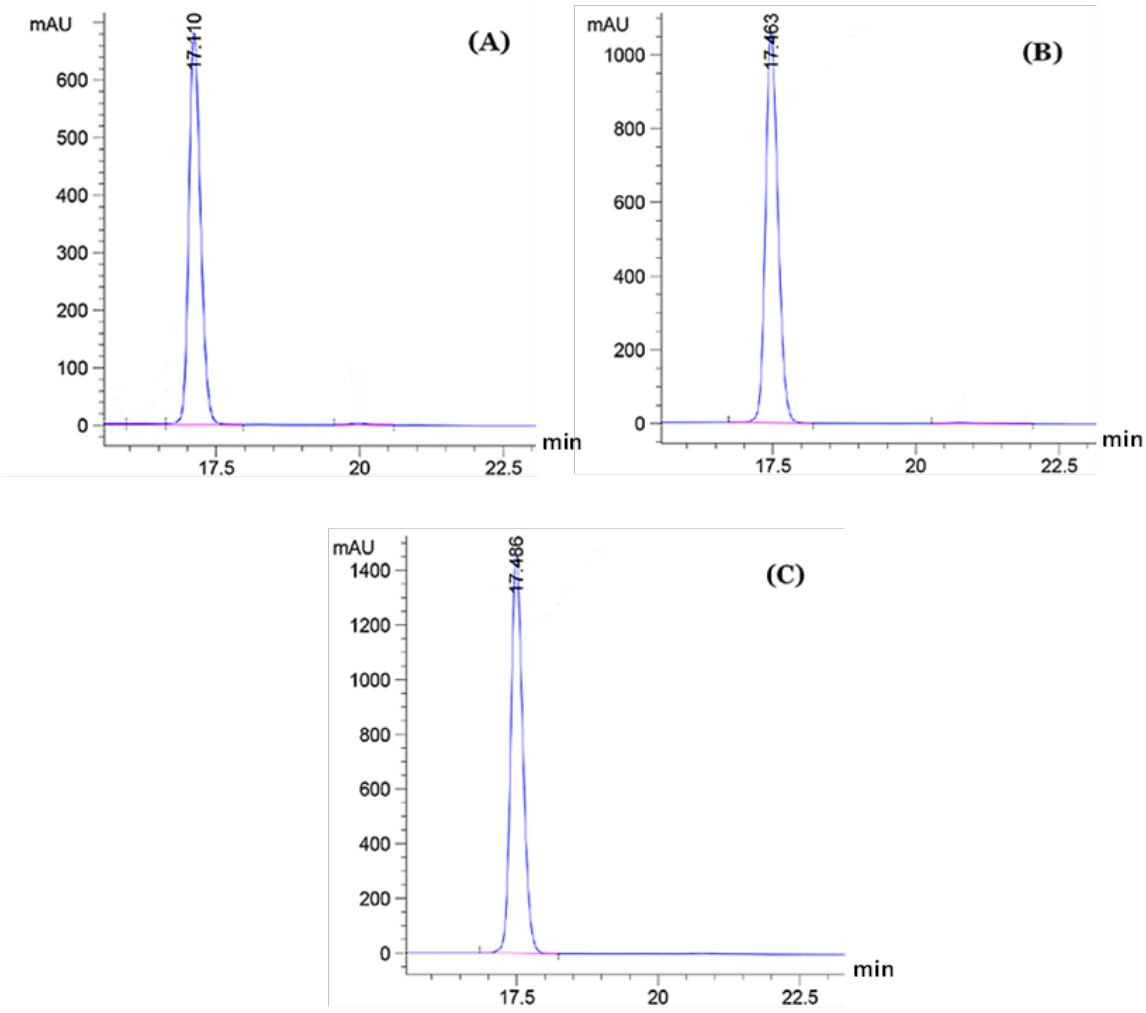

Figure 5. Representative chromatograms of dansyl chloride leftover from the reaction at different molar ratios of (A) 1:20, (B) 1:30 and (C) 1:40 showing retention times of 17.110 ± 0.009, 17.463 ± 0.009 and 17.486 ± 0.004 min, respectively

Fresh Garlic Derivatization with Dansyl Chloride

Garlic contains high amounts of nutrients and organic sulfur compounds [17]. Optimizing the analytical procedure for quantifying organosulfur compounds (OSCs), including S-allyl-cysteine in garlic, is one of the most critical procedures for content monitoring. Garlic samples should be derivatized using dansyl chloride to minimize interferences from background and side products.

A Previous study by Yoo et al. investigated flavor precursors in Allium plants and reported that using the simple and applicable dansyl chloride derivatization method, they were able to process a large number of samples, separate and quantify the individual S-alk(en)yl-L-cysteine sulfoxides (ACSOs) [18]. Therefore, we wanted to examine

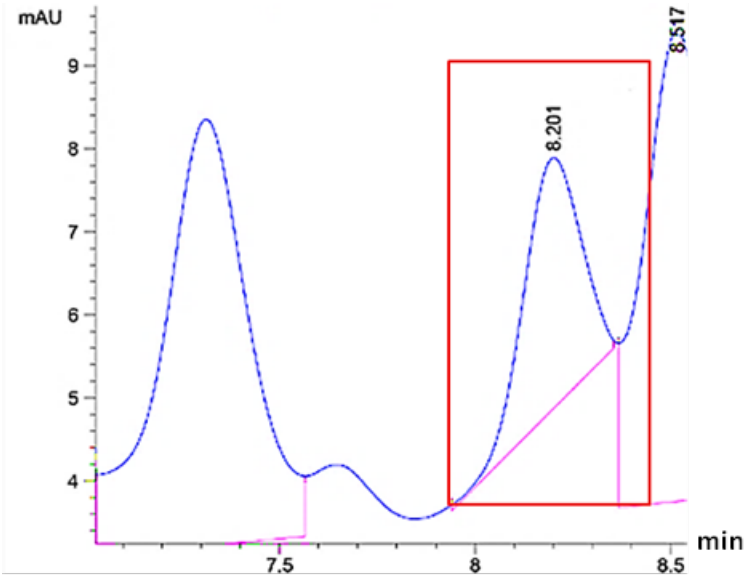

Figure 6. Representative chromatogram of S-allyl-cysteine in fresh garlic derivatized with dansyl chloride at the molar ratio of 1:20 showing retention time of 8.201 ± 0.038 min

whether the dansyl chloride method was also suitable for derivatization of S-allyl-cysteine, an interesting organosulfur compound from *Allium sativum* L. from fresh garlic and plant tissue culture in our study.

Chromatographic profiles and S-allyl-cysteine contents in fresh garlic derivatized with dansyl chloride at the molar ratios 1:20, 1:40, 1:60, 1:80 and 1:100 were compared. The HPLC of fresh garlic showed signal peak at a retention time of 8.201 ± 0.038 min (Figure 6) within the range obtained for the derivatized S-allyl-cysteine standard. The molar ratio of 1:20 (0.1285 ± 0.0002 µg/g) and 1:40 (0.1288 ± 0.0004 µg/g) did not result in significantly different S-allyl-cysteine contents. Moreover, slightly higher amounts of derivatized S-allyl-cysteine contents were detected for the molar ratios of 1:60 (0.1298 ± 0.0003 µg/g), 1:80 (0.1299 ± 0.0002 µg/g) and 1:100 (0.1299 ± 0.0005 µg/g) (Table 2). The dansylation molar ratio of 1:20 was appropriate and adequate for reaction with S-allyl-cysteine in fresh garlic. The Peak for S-allyl-cysteine in chromatogram was completely separated from peaks of other constituents.

Table 2. The molar ratio of S-allyl-cysteine from fresh garlic to dansyl chloride with derivatized S-allyl-cysteine content and amount of dansyl chloride leftover

Molar ratio of S-allyl-cysteine to dansyl chloride	Derivatized S-allyl-cysteine content from fresh garlic (µg/g)	Amount of dansyl chloride added to the reaction (µg)	Amount of dansyl chloride leftover from the reaction (µg)	Percentage of dansyl chloride leftover
1:20	0.1285 ± 0.0002 ^{b}	0.115	0.0243 ± 0.0003 ^{b}	21.30
1:40	0.1288 ± 0.0004 ^{b}	0.230	0.0258 ± 0.0004 ^{a}	11.21
1:60	0.1298 ± 0.0003 ^{a}	0.346	0.0227 ± 0.0003 ^{c}	6.56
1:80	0.1299 ± 0.0002 ^{a}	0.461	0.0217 ± 0.0002 ^{d}	4.70
1:100	0.1299 ± 0.0005 ^{a}	0.577	0.0227 ± 0.0001 ^{c}	3.93

All data in Table 2 are expressed as the mean ± standard deviation from triplicate determination. Different letters are multiple ranges significantly different reported by LSD test ($p < 0.05$)

The dansyl chloride leftover from reaction at the molar ratio of 1:40 (0.0258 ± 0.0004 µg), 1:60 (0.0227 ± 0.0003 µg), 1:80 (0.0217 ± 0.0002 µg), 1:100 (0.0227 ± 0.0001 µg) was mostly less than at the molar ratio of 1:20 (0.0243 ± 0.0003 µg). However, the molar ratio of 1:60 (0.1298 ± 0.0003 µg/g), 1:80 (0.1299 ± 0.0002 µg/g) and 1:100 (0.1299 ± 0.0005 µg/g) provided S-allyl-cysteine contents that were only slightly higher than at the molar ratio of 1:20 (0.1285 ± 0.0002 µg/g) and 1:40 (0.1288 ± 0.0004 µg/g) but were not significantly different. The amount of dansyl chloride added to the reaction at the molar ratio of 1:100 was 5 times higher than in the molar ratio 1:20, may not be worth. Although, dansyl chloride leftover at the molar ratio 1:100 was less than 1:20 (Table 2), their S-allyl-cysteine contents were not much

different. The molar ratio 1:20 was appropriate and adequate for reaction with S-allyl-cysteine content in fresh garlic.

The HPLC chromatogram of dansyl chloride leftover from the reaction with S-allyl-cysteine in fresh garlic showed signal peak at retention time of 17.173 ± 0.050 min (Figure 7) within the range obtained for dansyl chloride standard.

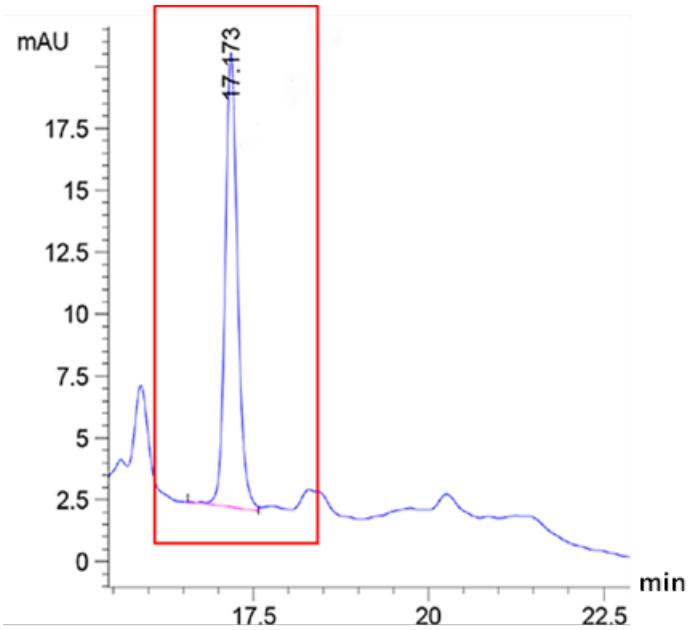

Figure 7. Representative chromatogram of dansyl chloride leftover in fresh garlic derivatized at the molar ratio of 1:20 showing retention time of 17.173 ± 0.050 min

Effect of 2,4- Dichlorophenoxyacetic acid on Production of S-allyl-cysteine in Garlic Callus

S-allyl-cysteine and various dipeptides serve as storage compounds while sulfoxides are the precursor of flavour in garlic. It has been reported that average aged garlic bulbs contain S-allyl-cysteine at about 0.45 mg/g and possess at least 30 times of S-allyl-cysteine higher than that found in fresh garlic [19]. Production of S-allyl-cysteine from aged garlic usually occurs after 2 years of storage. Our laboratory aimed to investigate the production of this compound through callus culture. Callus from plant tissue culture can be stimulated to increase the accumulation of bioactive compounds by addition of elicitors and precursors [20]. The use of elicitors is an effective strategy to increase bioactive compounds' productivity [21]. Moreover, previous studies found that growth regulators such as auxins and cytokinins successfully induced growth of calli on different garlic cultivars [22, 23]. Thus, it was our intention to explore the possibility that garlic callus will respond well to the regulator of choice and produce S-allyl-cysteine rapidly.

Garlic cloves were cultured in MS medium with 2,4-dichlorophenoxyacetic acid (2,4-D) at a concentration of 1.5 mg/L and kinetin at a concentration of 5.0 mg/L for 4 weeks (Figure 8A-8E). Elicitation of callus to produce S-allyl-cysteine was carried out with the same medium supplemented with 2,4-D at 0.05 mg/L for 8 weeks (Figure 8F). The protocol used for callus induction was that following the previous study by Priyanka and Muteba [24] where garlic callus was induced under fluorescent light for 16 hours of photoperiod at 2000-2500 lux. Setiowati et al. [20] and Priyanka and Muteba [24] also incubated garlic callus to produce organosulfur compounds using the same lighting conditions. However, callus from other plants have been induced and incubated in the dark. Pan et al. incubated *Bletilla striata* in the dark for accumulation of phenanthrenes while Park et al. cultured and maintained *Sophora flavescens* in the dark for production of flavonoids [25, 26]. After 2 weeks of culturing, callus appeared clearly around the new shoot emerging from the clove explant. Callus was subcultured after 4 weeks and grown for another 8 weeks. The obtained callus was compact with yellowish color.

Callus was weighted prior to extraction for HPLC analysis. It was demonstrated in this study that callus could be induced to manufacture S-allyl-cysteine sooner than in aged garlic, although this preliminary experiment may not provide as much S-allyl-cysteine as found in aged garlic.

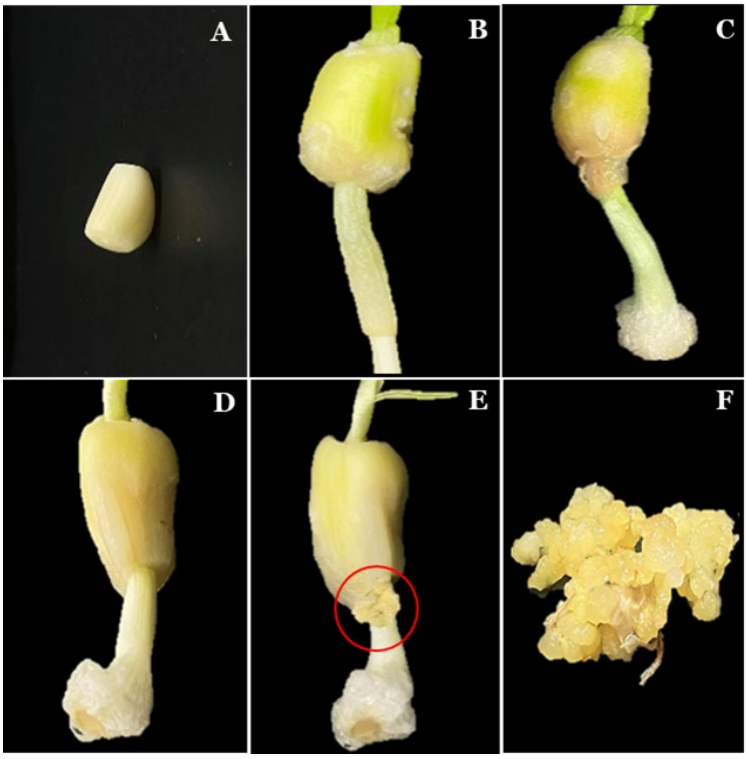

Figure 8. Production of callus from garlic cloves (A) Garlic clove explant, (B) 1 week old culture, (C) 2 weeks old culture, (D) 3 weeks old culture, (E) 4 weeks old culture with formation of callus indicated with the red circle, (F) 8 weeks old callus with yellowish color and compact characteristics

Derivatization of S-allyl-cysteine in Garlic Callus with Dansyl Chloride

The extract of callus garlic that was cultured in MS supplemented with 2,4-D at concentration of 0.05 mg/L revealed signal peak of derivatized S-allyl-cysteine with a clear separation from other peaks. The signal peak appeared at retention time of 8.218 ± 0.039 min (Figure 9) within the range of that obtained for S-allyl-cysteine standard.

Dansylation of S-allyl-cysteine in garlic callus was performed following those molar ratios performed on fresh garlic. There was no dansyl chloride leftover from the reaction for the molar ratio of 1:20 (Figure 10), when compared to those with higher (Table 3). In addition, the molar ratio of 1:20 (0.2331 ± 0.0008 µg/g) offered dansylated S-allyl-cysteine content higher than at the molar ratio 1:40 (0.2238 ± 0.0005 µg/g), 1:60 (0.1990 ± 0.0004 µg/g), 1:80 (0.1941 ± 0.0005 µg/g) and 1:100 (0.1823 ± 0.0002 µg/g) (Table 3). The S-allyl-cysteine present in garlic callus was derivatized with different molar ratios of dansyl chloride (Table 3). It was found that the molar ratio of 1:20 was the best as it provided the highest amounts of derivatized S-allyl-cysteine. Higher molar ratios resulted in lower amounts of derivatized S-allyl-cysteine were detected. The decrease in amounts detected regardless of higher amounts of dansyl chloride available for callus extract may be the result of dansyl chloride self-quenching effect. This phenomenon is observed here as in rather a hydrophilic environment dansyl chloride or dansylated S-allyl-cysteine, considered as hydrophobic molecules, may aggregate and come closer together at higher concentrations, in turn, affecting the UV absorption resulting in the lower signal. In fresh garlic sample which is more hydrophobic due to the presence of essential oils and higher lipid content, dansyl chloride molecules or dansylated S-allyl-cysteine are more distributed and, therefore, did not promote the self-quenching effect [12,13]. Consequently, the molar ratio of 1:20 was the best and dansylation sufficient for reaction with S-allyl-cysteine standard, S-allyl-cysteine present in fresh garlic and garlic callus. Furthermore, garlic callus apparently produced S-allyl-cysteine content about double of that found in fresh garlic, implying that garlic callus with optimized conditions is a potential source for S-allyl-cysteine production.

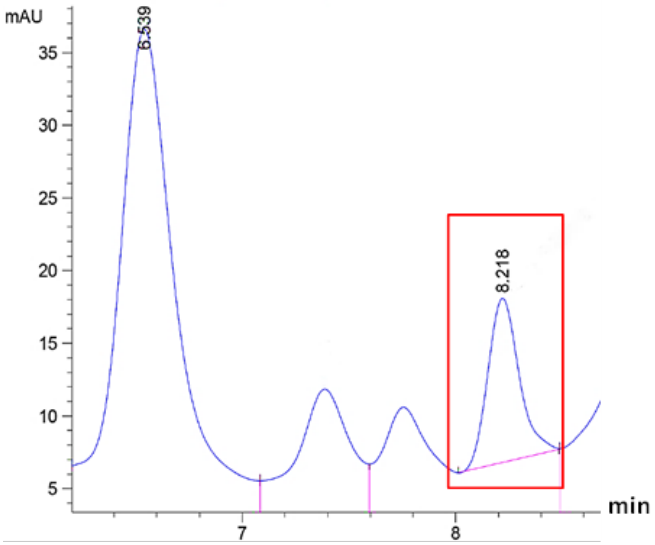

Figure 9. Representative chromatogram of S-allyl-cysteine in garlic callus derivatized with dansyl chloride at the molar ratio of 1:20 showing retention time of 8.218 ± 0.039 min

Table 3. The molar ratio of S-allyl-cysteine from garlic callus to dansyl chloride with derivatized S-allyl-cysteine content and amount of dansyl chloride leftover

Molar ratio of S-allyl-cysteine to dansyl chloride	Derivatized S-allyl-cysteine content from callus garlic (µg/g)	Amount of dansyl chloride added to the reaction (µg)	Amount of dansyl chloride leftover from the reaction (µg)	Percentage of dansyl chloride leftover
1:20	0.2331 ± 0.0008 [a]	0.115	0.0000 ± 0.0000 [e]	0.00
1:40	0.2238 ± 0.0005 [b]	0.230	0.0227 ± 0.0001 [a]	9.86
1:60	0.1990 ± 0.0004 [c]	0.346	0.0221 ± 0.0001 [b]	6.38
1:80	0.1941 ± 0.0005 [d]	0.461	0.0177 ± 0.0006 [c]	3.84
1:100	0.1823 ± 0.0002 [e]	0.577	0.0167 ± 0.0002 [d]	2.89

All data in Table 3 are expressed as the mean ± standard deviation from triplicate determination. Different letters are multiple ranges significantly different reported by LSD test ($p < 0.05$)

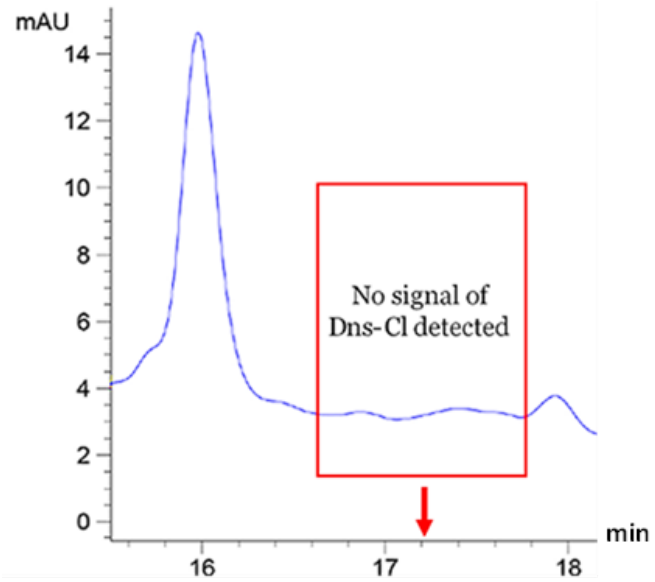

Figure 10. Representative chromatogram of dansyl chloride in garlic callus derivatized at the molar ratio of 1:20 no of Dns-Cl leftover at retention time of 17.353 ± 0.210 min

CONCLUSION

Derivatization of S-allyl-cysteine standard and S-allyl-cysteine present in fresh garlic and garlic callus with dansyl chloride at a molar ratio of 1:20 was the best to enhance detection sensitivity and improve the resolution with minimal dansyl chloride leftover while avoiding quenching effect. Moreover, the amount of S-allyl-cysteine found in garlic callus was approximately twice as much as that found the in native counterpart suggesting that with optimization plant tissue culture can be an alternative approach for S-allyl-cysteine production.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this manuscript.

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