

Quantification and Characterization of Allicin in Garlic Extract

Nurlidia Mansor, Ho Jian Heng, Sity Juairiah Samsudin, Suriati Sufian, and Yoshimitsu Uemura
Department of Chemical Engineering, Universiti Teknologi Petronas, Bandar Seri Iskandar, Perak, Malaysia
Email: {suriati, yoshimitsu_uemura, nurlidia_mansor}@petronas.com.my

Abstract—Garlic (*Allium sativum*) is a plant well known for its extensive use in traditional and modern medicine. Its healing properties are attributed to thiosulfonates, compounds which are formed through an enzymatic reaction when garlic cloves are crushed. This research studies the suitability of these compounds known as diallyl thiosulfonate or allicin to be used as a possible bio-inhibitor for urea fertilizers when subjected to local Malaysian soil conditions especially paddy fields. A spectrophotometric assay is used to quantify allicin by using L-cysteine with the theoretically proven basis that one molecule of allicin reacts rapidly with two molecules of cysteine to form two molecules of S-allyl mercaptocysteine. 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) is added to the mixture to form 2-nitro-5-thiobenzoate (NTB) after residual cysteine reacts with DTNB. Quantification of allicin is done at 30 °C with stock garlic extract concentration of 1 g/30 mL. This is followed by tests on allicin stability at pH 4.0 – 8.0 at 30 °C and thermal stability of allicin from 30 °C to 85 °C. The results show stability of allicin for local soil temperatures (30-35 °C) but unsuited for paddy field usage as it decomposes at pH lower than 6.0.

Index Terms—thiosulfonates, allicin, bio-inhibitor, urea fertilizer, *allium sativum*

I. INTRODUCTION

Thiosulfonates are not naturally present in an intact garlic bulb. Garlic bulbs have to be crushed in order to trigger the release of the enzyme alliinase to kick start the enzymatic reaction which produces thiosulfonates. Among the various types of thiosulfonates formed, allicin is produced in the greatest abundance. Besides its notable medicinal properties, allicin was also found to be a potential bio-inhibitor for urea fertilizers [1]. Due to environmental sustainability concerns bio-based inhibitors for agricultural use are becoming more sought after as they are more environmental friendly compared to the conventional chemical-based inhibitors. In the local scene however, bio-inhibitors have yet to take their place due to the lack of information on the suitability of the bio-inhibitors with the physical needs of local crops. The key parameters to study are the pH and temperature tolerance of allicin as these parameters have been known to affect the stability of allicin directly by causing decomposition to take place. Thus, the objectives of this research are to quantify allicin and study its stability at a

chosen range of temperature and pH which reflects local soil conditions particularly in paddy fields.

A. Allicin in Garlic Extract

Alliinase converts naturally occurring organosulfur compounds, particularly γ -glutamylcysteines and cysteine sulfoxides into thiosulfonates. This is a two-step reaction whereby pyruvic acid, ammonia and alk(en)yl sulfenic acid are formed as intermediates. Sulfenic acid will then undergo rapid condensation to form thiosulfonates, R-S(O)-S-R1. Among the eight types of thiosulfonates found in garlic extract, allicin has the greatest abundance at 50 – 90 mol% [2]-[3]. Comparatively, garlic has greater total sulfur compound (1.1 – 3.5%) than onions (0.4 – 1.2%) [4]. Hence, garlic provides greater amount of thiosulfonates per unit weight.

B. Quantification Methods

The conventional methods for quantifying thiosulfonates are using High Performance Liquid chromatography (HPLC) and Gas Chromatography (GC). However, both of these methods require external standards which have very low resolution. For GC in particular, the unstable thiosulfonates have to be converted to more stable compounds which are able to withstand high temperatures within the GC unit. Past researchers have also used spectrophotometric methods to quantify thiosulfonate. Using a direct approach, thiols are used to react with the disulfide bond present in thiosulfonates and the resulting thiol concentration is monitored. For an indirect approach, the excess thiol is reacted with a disulfide acid compound 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) [5]. The reaction forms a yellow colored compound that is detectable in the visible ultraviolet (UV) range. Another method combines both spectrophotometry and chromatography to specifically quantify allicin. This analytical method uses reversed phase HPLC with UV and electrochemical detection (ED) coupled with on-line post-column photochemical reaction. Since allicin is electrochemically inactive, post-column irradiation at 254 nm reduces the responsiveness of allicin to UV detector but allow it to be detected using electrochemical detector. This method saves time as less sample preparation steps are required and therefore have higher success rate of achieving desired results.

C. Soil pH and Temperature.

The pH value for West Malaysian paddy fields has a mean value of 4.7, with pH 3.4 as the minimum and pH

6.1 as the maximum [6]. The acidic nature of the local paddy soil is largely due to the leaching of fertilizers into the ground which is facilitated by humid weather and swampy areas with high levels of unsaturated organic matter. Most paddy plants within this region thrive in soil pH 5.5 [7]. According to the Malaysian Meteorological Department (2014), the highest recorded temperature in Malaysia to date is 40.1 °C whereas the average ambient temperature for lowlands in 2014 is 28 °C to 30 °C [8]. The optimal pH for thiosulfates at 37 °C is tabulated in Table I [10]. On the other hand, research reported that thiosulfates are unaffected by acidic conditions as well as extreme temperatures up to 100 °C [9]. Another research reveals that thiosulfates with longer and saturated alk(en)yl groups are more stable than thiosulfates with shorter, unsaturated alk(en)yl groups [11]. A test on thiosulfate decay conducted from pH 1.2 – 9.0 and at 20 °C to 80 °C revealed that the pH trend in decreasing order is best for thiosulfate stability.

TABLE I. OPTIMAL FORMATION pH FOR VARIOUS THIOSULFINATE

Thiosulfinate	Optimal pH range
Allicin, 1-propenyl allyl, allyl 1-propenyl	4.5 – 5.0
Allyl methyl, methyl allyl 1-propenyl methyl, methyl 1-propenyl	6.5 – 7.0
dimethyl	5.5

II. METHODOLOGY

A spectrophotometric assay developed by Han *et al.* (1995) is used to quantify allicin in all of the experiments. The garlic used to prepare garlic powder is of China origin. In preparing 1g/30mL of garlic extract, 5 g of garlic powder is dissolved in 150 mL of deionized water. The mixture is homogenized in an incubated shaker at 150 rpm at 23 °C. Insoluble solids are filtered off and the extract is centrifuged at 250 mg for 10 minutes. The final extract is obtained after filtration. For quantification tests, 0.5 mL of garlic extract is added to 1.2 mL of 2mM L-cysteine and allowed to sit at 30 °C for 10 minutes. Then, 3 mL of 50mM HEPES buffer (pH 7.6) and 1 mL DTNB is added to the solution where it is then hand shaken and allowed to sit for 2 minutes at room temperature. Absorbance of the samples are read at 412 nm with Perkin Elmer Lambda 25 UV-Vis Spectrophotometer. A molar absorptivity of 14150 and Beer-Lambert's Law is used to obtain the concentration of excess L-cysteine remaining in the sample.

III. RESULTS AND DISCUSSION

A. Quantification of Allicin at 30 °C.

From the results obtained, it was observed that allicin concentration increases with the increase in garlic extract. A linear fit line with regression squared value of 0.88 is obtained. This shows that there is a linear increase in allicin content for every increase in garlic extract concentration. In the quantification process, for each mole of unreacted L-cysteine remaining, one mole of

NTB is formed on reaction with DTNB. The concentration of NTB formed is determined through absorbance reading at 412 nm with molar absorptivity of 14150 for NTB anion [5]. When the amount of NTB present is known, the amount of allicin originally present in the sample is therefore back-calculated. Since this method quantifies the total thiosulfinate present in garlic extract, a factor of 0.7 is used to obtain the amount of allicin present, referring to the fact that allicin makes up 60 – 80 % of thiosulfates present in garlic extract [9]. From the plot as shown in Fig. 1, a straight line equation was obtained.

$$Y = 0.0028X + 0.5998 \quad (1)$$

where,

Y = Allicin content (mM)

X = Garlic extract concentration (g/mL)

This correlation is only applicable to a maximum garlic extract concentration of 1g/30mL. It is also restricted to quantification of allicin in fresh garlic extract at temperature of 30 °C due to the assumption that allicin decomposes at different rate in different temperature conditions.

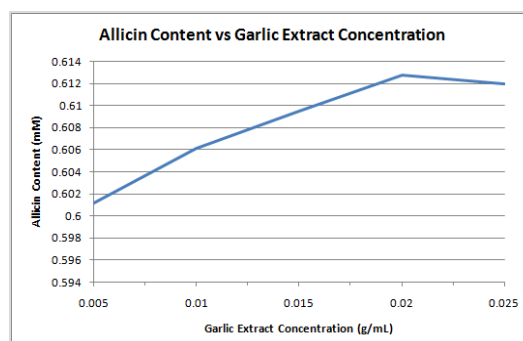


Figure 1. Plot of allicin content versus garlic extract concentration

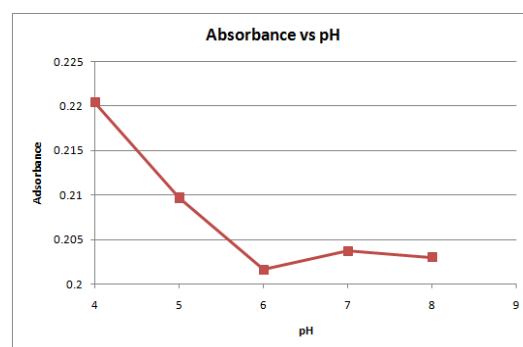


Figure 2. Plot of absorbance vs. pH

B. Effects of pH on Allicin at 30 °C.

The spectrophotometric assay conducted by Han *et al.* (1995) provides an indirect way of measurement to allicin content. A low absorbance reading signifies low amount of NTB present and consequently, low amount of leftover cysteine. Hence, low absorbance reading represents high amount of allicin present. The results as shown in Fig. 2 reflect the reduction of allicin at pH 4 and 5. Allicin tends to decompose when subjected to acidic conditions compared to alkaline conditions. This is due to allicin's

low tolerance to the acidic environment due to the presence of nucleophiles. The disulfide bond within allicin is highly susceptible to nucleophilic attacks [12]. Thus, when the S-allyl bond in the R-S(O)S-allyl compound is split, allicin decomposes. The plot in Fig. 2 also shows that the compound is fairly stable within the pH range of 6 through 8. Thus, alkaline conditions between these ranges are believed to have less effect towards allicin compared to lower pH values [13], [14].

C. Effects of Temperature on Allicin.

As shown in Fig. 3, allicin shows that it is most stable at temperature of 30 °C and only a slight reduction of 0.002 mM of allicin observed at 35 °C. At 40 °C however, the compound shows a more prominent but gradual decomposition trend, decomposing at a rate of 0.027/min. An almost immediate decomposition of allicin was then observed at a much higher temperature of 85 °C. Allicin content reduced 11mM in the first 60 minutes. As an organic compound, allicin is susceptible to decompose at higher temperature. A disulfide bond (S-S), typically has a bond dissociation energy of 250 kJ/mol where when a higher temperature is maintained, more heat energy is supplied into the system which allows the bond's dissociation energy to be overcome easily. As the disulfide bond present in allicin is 40% weaker than C – C and C – H bonds (Shin *et al.*, 2014), the disulfide bond is the weakest link in the allicin structure which is most susceptible to cleavage when higher heat is supplied [14].

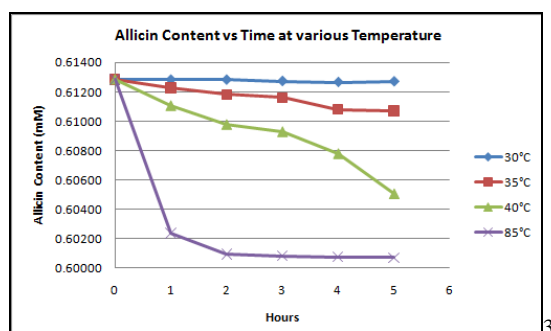


Figure 3. Allicin content vs. time at various temperature

IV. CONCLUSIONS AND RECOMMENDATIONS

This study looks into the stability of allicin at different pH and temperature ranges to allow a further understanding of the suitability of allicin to be used as a bio-inhibitor in urea fertilizers. The outcome of the experiments shows that allicin is unstable at lower pH and more stable at pH 6.0 to 8.0. Allicin also shows an almost stable condition with slow decomposition rate within the temperature range of 30 °C to 35 °C. However, at 40 °C the compound starts to show gradual decomposition. This shows the suitability of possibly applying allicin as a bio-inhibitor source for local fertilizer as it is within the ranges of local soil temperature. However, the processes and storage of the compound in future production for incorporation in urea fertilizer must not go beyond 40 °C as it will begin to decompose as shown in the study. In terms of pH ranges

however, allicin show better stability at pH higher than 6.0. This will thus challenge the suitability for usage of allicin in local soil as the nature of paddy fields in particular, is mostly acidic. The incorporation of allicin in urea fertilizer will therefore have to be modified to be able to withstand pH conditions of the soil with possible coating elements within the fertilizer. Overall, these parameters provide an insight on the characterization of allicin for the integration as bio-inhibitor into urea fertilizers for use in the local scene.

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Nurlidia Mansor is a senior lecturer at the Department of Chemical Engineering, Universiti Teknologi PETRONAS, Malaysia. She holds a BSc (Hons) in Biotechnology and an MSc in Environmental Engineering from Universiti Putra Malaysia. Dr. Mansor received her PhD in Environmental Chemistry from Glasgow University, United Kingdom in 2009. Her research interest lies in the areas of biomonitoring focusing on heavy metals from

anthropogenic sources as well as former mining activities. She also conducts bioremediation research using phytoremediation technology as clean-up solutions to contaminated soil and water bodies. Dr. Mansor also works with microalgae as green biosorbents for wastewater treatment. At the university, Dr. Mansor is also part of a research group that studies alternative green materials for improvements in agricultural technology. She is currently an associate member of IEEE and an affiliate member of IChemE.



Suriati Sufian is an Associate Professor at the Department of Chemical Engineering, Universiti Teknologi PETRONAS, Malaysia. She holds a BEng (Hons) in Chemical Process Engineering with Fuel Technology from University of Sheffield, UK and an MEng in Chemical Engineering from University of Queensland, Australia. Dr. Sufian received her PhD in Chemical Engineering from Universiti Teknologi PETRONAS (UTP), Malaysia.

Currently, her research works include synthesis and characterization of advanced materials such as carbon nanomaterials, nanocatalyst and hydrotalcite. Most of the synthesized materials are applied in catalysis

including catalyst support, adsorbent in waste water treatment (dye and heavy metal removal), CO₂ capture, H₂ storage, H₂ production and many more. Dr Sufian is currently the Head of Chemical Engineering department at UTP and an associate member of IChemE.



Prof Dr. Yoshimitsu Uemura obtained his higher education at Tokyo Institute of Technology where he was awarded with a BSc in Chemical Engineering in 1977, Masters of Science in 1979 and his Doctor of Philosophy in 1990. Prof Uemura became an Associate Professor at Kagoshima University and during his work at Kagoshima University, he was later assigned as a visiting researcher and postdoctoral fellow at University of

Western Ontario and Toronto, Canada, respectively. Prof. Uemura is currently the Mitsubishi Chair and heads the Centre for Biofuel and Biochemical Research at Universiti Teknologi PETRONAS, Malaysia. Prof Dr. Yoshimitsu Uemura is actively involved in many research projects in the field of chemical engineering including catalytic reaction engineering, reactor engineering and polymer engineering and their application to the biomass conversion, energy and environmental issues.