QUANTIFICATION AND CHARACTERIZATION OF ALLICIN IN GARLIC EXTRACT

by

HO JIAN HERNG
15096

Dissertation submitted in partial fulfilment of
the requirements for the
Bachelor of Engineering (Hons)
(Chemical)

MAY 2014

Universiti Teknologi PETRONAS

Bandar Seri Iskandar

31750 Tronoh

Perak Darul Ridzuan

CERTIFICATION OF APPROVAL

Quantification and Characterization of Allicin in Garlic Extract

by

Ho Jian Herng
15096

A project dissertation submitted to the

Chemical Engineering Programme

Universiti Teknologi PETRONAS

in partial fulfilment of the requirement for the

BACHELOR OF ENGINEERING (Hons)

(CHEMICAL)

Approved by,

……

(DR. NURLIDIA MANSOR)

UNIVERSITI TEKNOLOGI PETRONAS

TRONOH, PERAK

May 2014

CERTIFICATION OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this project, that the original work is my own except as specified in the references and acknowledgements, and that the original work contained herein have not been undertaken or done by unspecified sources or persons.

……

(HO JIAN HERNG)

ABSTRACT

Garlic (*Allium sativum*) is a plant well known for its extensive use in traditional and modern medicine. Its healing properties are attributed to thiosulfinates, compounds formed through an enzymatic reaction when garlic cloves are crushed. One of the most prominent thiosulfinate found in garlic is diallyl thiosulfinate or allicin.

The condition in this research mimics Malaysian climate conditions. This allows allicin to be studied and characterized for its uses in the local agricultural sector as potential urea inhibitor. A spectophotometric method is used in this research to quantify allicin found in garlic extract. L-cysteine is used to react with allicin in garic extract with the knowledge 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 so that spectrophotometrically active 2-nitro-5-thiobenzoate (NTB) is formed after residual cysteine reacts with DTNB. Quantification of allicin is done at 30 ℃ with garlic extract concentration ranging from 0.005 g/mL to 0.03 g/mL. This is followed by tests on allicin stability at pH 4 – 8 at 30 ℃ and effects of temperature on allicin from 30 ℃ to 85 ℃.

ACKNOWLEGDEMENT

In completion of this final year project, I would like to thank Universiti Teknologi PETRONAS for providing the facilities and a good environment to conduct this study. I would also like to extend my gratitude towards Dr, Nurlidia Mansor for her guidance and support throughout the project. Besides, I would also like to extend my appreciation to Miss Sity Juaeiriah Binti Samsudin for her guidance and experience shared on studies related to this field. I would also like to thank the lab technicians for their cooperation and support while using the laboratory. Lastly, I would like to thanks my family for their continual support that drove to complete this project.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Thiosulfinates is an active ingredient found after garlic cloves are crushed due to an enzymatic reaction which involves the enzyme, allinase. The thiosulfinate, allicin is formed in the greatest amount among all thiosulfinates formed. Recent research has shown that thiosulfinates from garlic extract has the potential to act as inhibitors to the urea enzymatic reaction which occurs in urea fertilizers (Juszkiewicz et al., 2003). The presence of environmental friendly urea inhibitors such as thiosulfinates could prevent ammonia released from the urea fertilizer reaction. Hence, the nature of thiosulfinates derived from garlic extract has to be understood so that researchers could exploit its inhibition properties. This research project aims to quantify and characterize a specific thiosulfinate found in garlic extract which is allyl-2-propenethiosulfinate (allicin). The experiments in this research project will be tailored made to suit conditions in local paddy fields so that its results are applicable in similar researches. Various quantification techniques have been employed by various researchers to quantify thiosulfinates found in garlic extract. Researchers have also performed studies on thiosulfinate formation rate in various pH. However, there is limited information regarding thermal stability of thiosulfinates. The experiments involved will be conducted using Ultraviolet Visible Spectroscopy (UV-VIS).

1.2 Problem Statement

Quantification of thiosulfinates found in garlic extract is not a straight forward process because a simple and accurate method is yet to be developed. Also, thiosulfinate content varies according to region. Hence, quantification of thiosulfiantes of a fixed source can be done by formulating the relationship between amounts of garlic extract to amount of allicin yield.

On the other hand, characterization of thiosulfinates is mostly done according to conditions in the human body due to it having antioxidant, antimicrobial and anti-inflammatory properties. Hence, there is limited information on allicin characterization suited to agricultural needs. Characterization in terms of allicin's physical stability at various temperatures and pH suited to standard Malaysian soil conditions will promote greater use of allicins in the local agricultural sector.

1.3 Objectives

The main objectives of this research project are:

- To develop an equation which relates allicin content to amount of garlic extract at 30 ℃.
- To characterize the stability of allicin at different temperatures and different pH at 30 ℃.

1.4 Scope of Study

The experiments will use garlic extract derived from garlic of China origin as the source of allicin. All experiments will include the use of cysteine as an indicator of allicin content due to the reactivity of thiosulfinates to free –SH groups (Qi & Wang, 2003). One molecule of allicin reacts rapidly with two molecules of cysteine to form two molecules of S-allyl mercaptocysteine (Han et al., 1995). Beer-Lambert's law will be utilized to determine allicin content in each experiment. The experiments involved in this research project are:

- Quantification of allicin at 30℃.
- Effects of pH on allicin at 30 ℃.
- Effects of temperature on allicin.

1.5 Relevancy of Project

This project is significant as it addresses the issue faced by ammonia fertilizers, which is its rapid rate of leaching into the soil and inhibitors are required to slow down this leaching rate. The use of allicin as a bio-inhibitors provides an edge over chemically derived inhibitors because they are sustainable and bio-degradable.

1.6 Feasibility of Project

This project is feasible in the given time frame of 2 semesters as it only covers 3 aspects. The required equipments, chemicals and laboratory facilities are readily available. Thus, this allows the project to proceed immediately after proposal.

CHAPTER 2

LITERATURE REVIEW

2.1 Thiosulfinates (TS) in Garlic Extract

Garlic (Allium sativum) is a plant that has been widely used in traditional medicine for thousands of years (Juszkiewicz et al., 2003). Due to its unique pungent flavour, it is used to prepare food in various parts of the world. Intact garlic bulbs contain organosulfur compounds, particularly γ-glutamylcysteines and cysteine sulfoxides (Olech & Zaborska, 2012). The enzyme, alliinase catalyses the formation of thiosulfinates when garlic cloves are crushed through the reaction scheme below:

$$R\text{-}S(O)\text{-}CH_2\text{-}CH(NH_2)\text{-}COOH + H_2O \rightarrow R\text{-}SOH + CH_3\text{-}C(O)\text{-}COOH + NH_3$$

$$R\text{-}SOH + R_1\text{-}SOH \rightarrow R\text{-}S(O)\text{-}S\text{-}R_1 + H_2O$$

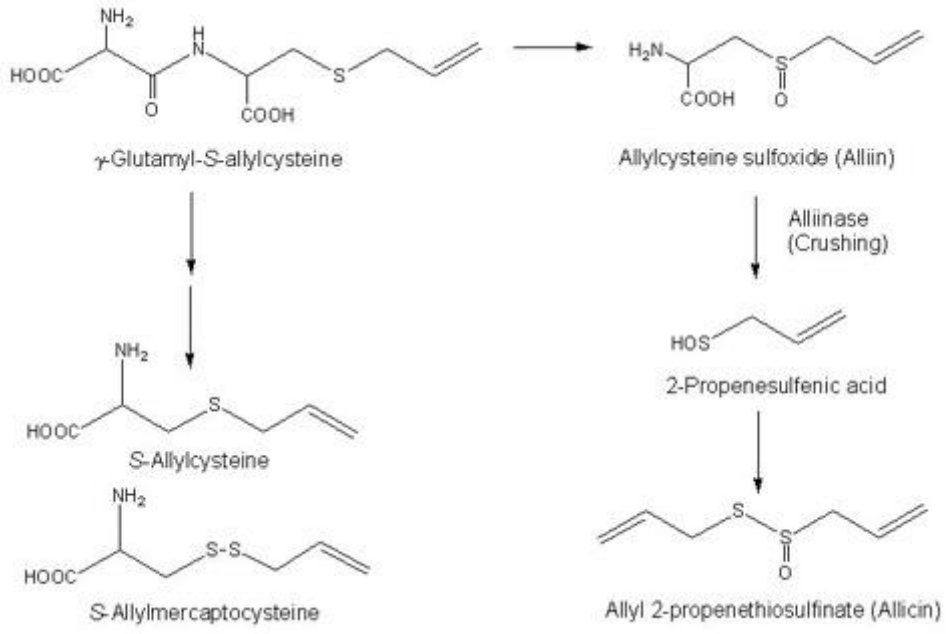


Figure 2.1: Formation path of allicin

Initially, pyruvic acid, ammonia and alk(en)yl sulfenic acid are formed. Then, sulfenic acid undergoes rapid condensation to form thiosulfinates $R\text{-}S(O)\text{-}S\text{-}R_1$. There are eight known dialk(en)yl thiosulfinates in crushed garlic (Kyung & Lee, 2001).

Table 2.1: Types and concentration of thiosulfinates found in garlic extract

Thiosulfinate (TS)	**%mol**
Allyl-2-propeneTS (allicin)	50 – 90
AllylmethaneTS	3 – 20
trans-1-propenyl-2-propeneTS	5 – 18
methyl-2-propeneTS	1.5 – 8
Allyl-trans-1-propeneTS	1.5 - 2
methylmethaneTS	1 – 2
Trans-1-propenylmethaneTS	1 - 2
methyl-trans-1-propeneTS	0.5

2.2. Thiosulfinate Qunatification Methods

Despite allicin, allinase and the reaction to form TS has been discovered and examined since 1944 (Cavallito & Bailey, 1944), a simple and sensitive method of quantitative TS determination is yet to be uncovered (Olech & Zaborska, 2012). Some researchers such as Rosen et al. and Arnault et al. made use of GC and HPLC methods to quantify TS. However, if GC is to be used, allicin and other thiosulfinates must be converted to more stable compounds, which are less sensitive to high temperature as TS. Both GC and HPLC are limited by its low resolution and their need to use external standards (Olech & Zaborska, 2012). On the other hand, Keusgen et al. (2003) applied the enzymatic biosensors method to quantify TS. This method is quick and precise and does not require complex equipment, however, the enzyme is difficult to obtain.

Spectrophotometric method provides two methods to quantify TS; they are the direct method and indirect method. In direct method, thiols (compounds with sulphydryl groups, -SH) are used since they react with a disulphide bond of TS. Thiol concentration is observed to determine the course of reaction. This method is used by Miron et al. On the other hand, Han et al. (1995) uses the indirect method whereby disulfide acid compound 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) is

used. TS is reacted with an excess of cysteine and remaining cysteine is reacted with DTNB. The reaction forms a coloured compound that is detectable in the visible UV range.

Apart from that, Yoo & Mike (2001) quantified TS by monitoring ammonia and pyruvic acid content during the enzymatic reaction. The limitation to this method is that both ammonia and pyruvic acid exist even before the garlic cloves are crushed. Thus, background concentration of these two components has to be accounted for this method to be valid. Olech & Zaborska (2012) used a new method for TS quantification that exploits TS reactivity with chromogenic thiols. Knowing the λ_{max} of chromogenic thiol used allows tracking its reaction with TS and at the same time, determining the reaction's rate constant.

On the other hand, Bocchini et al. (2001) combined both spectrophotometry and chromatography to quantify thiosulfinate, specifically, diallyl thiosulfinate (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 reduced the responsiveness of allicin to UV detector but allowed it to be detected using electrochemical detector. This method saves time as less sample preparation steps are required.

2.3 Malaysian Soil pH and Ambient 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 (Kawaguchi & Kyuma, 1974). 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. The paddy plant thrives in soil pH 5.5 (Yu, 1991). According to the Malaysian Meteorological Department (2014), the highest

recorded temperature in Malaysia to date is 40.1℃ whereas the average ambient temperature for lowlands in 2014 is 28℃ to 30℃.

2.4 Effects of pH and Temperature on Thiosulfinates

The effects of pH on yield of dialkyl thiosulfinates released from garlic powder and garlic extract were studied by Lawson & Hughes (1991). Table shows the respective thiosulfinates with their optimum formation pH. The pH tests were carried out at 37℃.

Table 2.2: Optimal pH for various thiosulfinate yields

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

On the other hand, Shen et al. (2002) characterized thiosulfinate decay as a first-order process over the pH range of 1.2 – 9.0 at 20 - 80°C. Their experimental work also showed pH ranges that thiosulfinate remained stable and thiosulfinates with longer and saturated alk(en)yl groups are more stable than thiosulfinates with shorter, unsaturated alk(en)yl groups. The order of stability in decreasing order:

pH 4.5 – 5.5 > pH 1 – 2 > pH 6.5 – 7.5 > pH 8.0 – 9.0

Lawson & Hughes (1991) also performed rate of formation formation tests on thiosulfinates at pH 5.6. It was found that the time required to release each thiosulfinates to their maximum amounts were independent of their incubation temperature. However, they did not study the stability of the thiosulfinates formed at

various temperatures. On the other hand, Sariri et al. (2002) studied the stability of allicin from garlic and garlic products of Iranian origin. They found out that allicin formation and stability was unhindered when exposed to denaturing conditions, particularly, acidic and high temperature conditions. The possible explanation to this phenomenon is the presence of heavy metals in the extract that might shield the enzyme alliinase from being denatured.

CHAPTER 3

METHODOLOGY

3.1 Project Flow Chart

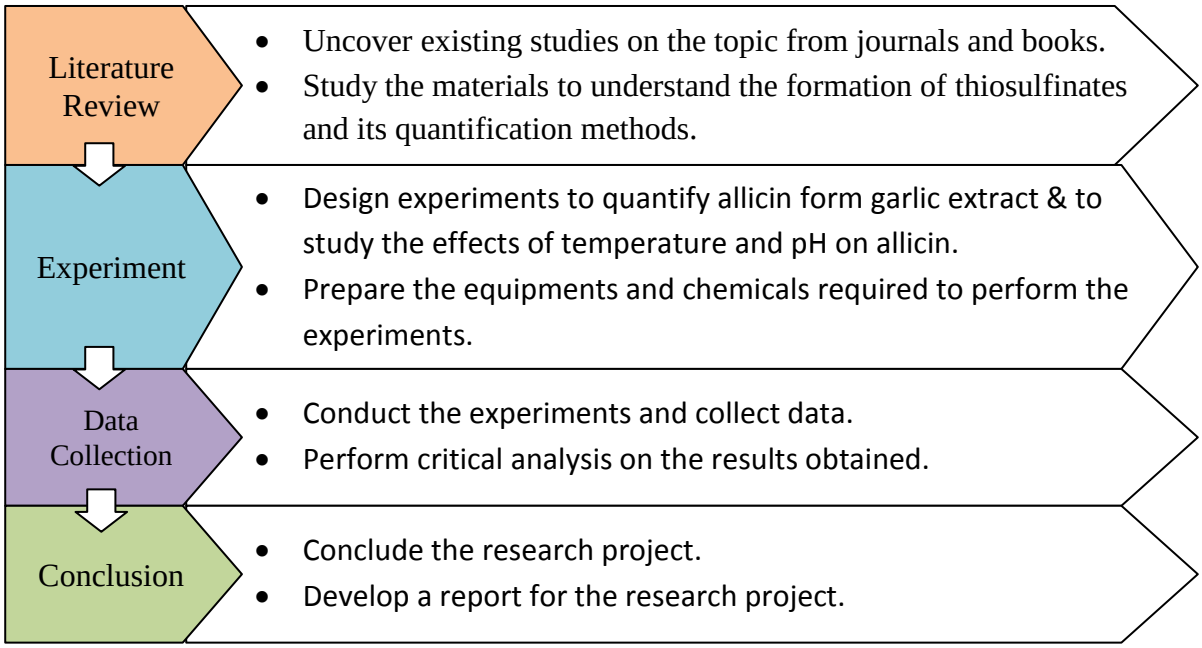
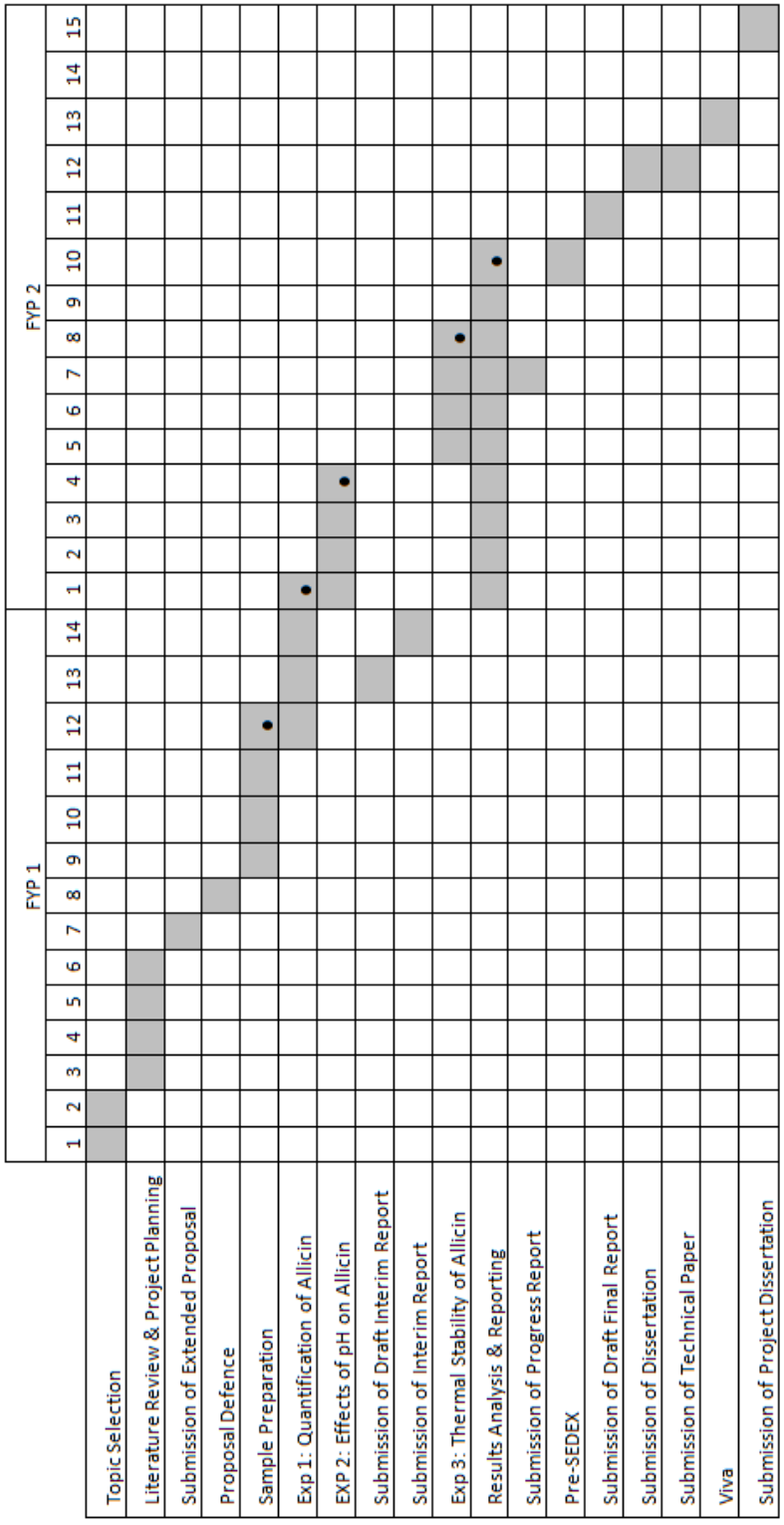


Figure 3.1: Project flow chart

3.2 Gantt Chart and Key Milestones

Table 3.1: Project Gantt chart and key milestones



- Key Milestones

3.3 Experimental Methodology

For the purpose of this project, the Ultraviolet – Visible (UV-Vis) Spectrophotometry indirect method used by Han et al. (1995) is adopted as the main experimental method. This method uses excess L-cysteine to react with allicin and other thiosulfinates present in the garlic extract. Remaining L-cysteine in the sample will be reacted with 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) with the presence of pH 7.6 HEPES buffer. Excess L-cysteine will react with DTNB at a stoichiometry of 1:1 mole to yield 2-nitro-5-thiobenzoate (NTB). NTB is yellow in colour and it is spectophotometrically active in the UV region at 412 nm. To analyse the absorbance reading, Beer's Law and molar absorptivity of 14150 is used to get the concentration of excess L-cysteine remaining in the sample.

Beer's Law:

$$A = \varepsilon bc$$

Where, A = Absorbance

ε = Molar absorptivity [L mol^{-1} cm^{-1}]

b = Path length [cm]

c = Concentration of NTB [mol L^{-1}]

This method is preferred over more conventional methods such as HPLC and GC because it is difficult to obtain and synthesize the allicin standard at the given timeframe. Due to the instability of pure allicin, the allicin standard has to be synthesised right before the experiment starts. Conveniently, the Han method does not require a standard to measure the amount of allicin present.

3.3.1 Preparation of Garlic Extract (GE)

The garlic used throughout this project will originate from China. This ensures consistency in thiosulfinate concentration in the garlic extract. Garlic extract was prepared by dissolving garlic powder in deionized water. To homogenize the

mixture, the garlic solution is placed in an incubated shaker for 1 hour. The incubated shaker is set at 150 RPM and 23°C. The homogenized mixture is then filtered using a coffee sieve in order to remove remaining large particulates. Then, the filtered solution is centrifuged at 250 xg for 10 minutes. After that, the centrifuged solution is filtered using Whatman Ø150mm (Cat No 1441 150) circular filter paper to yield the final garlic extract.

3.3.2 Preparation of 2.0 mM L-cysteine solution

0.0606 g of L-cysteine crystal is weighed using a mass balance. The crystals are transferred into a 250 mL Erlenmeyer flask and 250 mL of deionized water is added into the flask. The solution is mechanically shaken in the incubated shaker for 1 hour at 150 RPM at 23°C for homogenization.

3.3.3 Preparation of 5.0 mM DTNB solution

0.1982 g of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) powder is weighed using a mass balance. The weighed powder is transferred into a 100 mL Erlenmeyer flask and dimethyl sulfoxide (DMSO) is added until the volume reaches the 100 ml level. The solution is mechanically shaken in the incubated shaker for 1 hour at 150 RPM at 23°C for homogenization.

3.3.4 Preparation of 50 mM HEPES buffer

5.0 mL of 1.0 M HEPES buffer is introduced into an empty 150 mL beaker. 100 mL of deionized water is added to the beaker. The solution is mechanically shaken in the incubated shaker for 1 hour at 150 RPM at 23°C for homogenization.

3.3.4 Tools and Equipment

This experiment requires an UV-Vis spectrophotometer (Perkin Elmer Lambda 25) for absorbance reading to monitor allicin concentration. Apart from that,

hot plates and water bath set ups are required to maintain constant temperature for allicin thermal stability test. An incubated shaker is also required to homogenise prepared solutions. A pH meter is required to measure the pH of samples.

3.3.5 Substance and Chemicals

The chemicals required for this project are:

Garlic powder, deionized water, 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB), dimethyl sulfoxide (DMSO), L-cysteine, 1M HEPES buffer (pH 7.6), 1.0 M hydrochloric acid and 1.0 M sodium hydroxide solution.

3.3.6 Proposed Experimental Procedure

3.3.6.1 Quantification of Allicin in Garlic Extract

i. Prepare 5 g/150 mL of garlic extract (GE) stock solution by dissolving 5.0 g of garlic powder in 150 mL of deionized water. Standard preparation and filtration procedures were followed.
ii. From the stock solution, 5 mL samples of concentration 0.005 g/mL, 0.010 g/mL, 0.015 g/mL, 0.02 g/mL and 0.025 g/mL garlic extract were prepared using dilution with deionized water.
iii. 0.5 mL of each sample is transferred into separate beakers and 1.2 mL of 2 mM L-cysteine is added into each sample. The samples are allowed to sit for 10 minutes at room temperature.
iv. For each sample, 3 mL of 50 mM HEPES buffer and 1 mL of 5 mM DTNB is added. The samples are swirled and allowed to sit at room temperature for 2 minutes.
v. For absorbance reading, each sample is transferred into respective cuvettes and the absorbance is read at 412 nm using a UV-Vis spectrophotometer. By measuring the amount of NTB formed at molar absorbance of 14151 at 412 nm, the remaining concentration of L-cysteine is determined.

3.3.6.2 Effects of pH on Allicin Formation

i. Prepare 5 g/150 mL of garlic extract (GE) stock solution by dissolving 5.0 g of garlic powder in 150 mL of deionized water. Standard preparation and filtration procedures were followed.
ii. The pH of the stock solution is determined using a pH meter.
iii. 5.0 mL of stock solution is placed into 5 respective beakers with 1 labelled as control. The pH of 4 remaining samples is adjusted using 1.0 M hydrochloric acid and 1.0 M sodium hydroxide solution.
iv. Samples of pH 4, 5, 7 and 8 are prepared.
v. The samples are allowed to incubate at 30°C for 10 minutes.
vi. 0.5 mL of each sample is extracted and placed into separate empty beakers. 1.2 mL of 2.0 mM L-cysteine is added to each sample and they are allowed to sit at room temperature for 10 minutes.
vii. 3.0 mL 50 mM HEPES buffer and 1.0 mL of DTNB solution is added into each sample. The respective samples are swirled and allowed to sit at room temperature for 2 minutes.
viii. The samples are transferred into separate cuvettes. The absorbance of the samples is read at 412 nm using a UV-Vis spectrophotometer. The amount of NTB formed in the mixture at could be used to determine amount of remaining cysteine in the mixture by using molar absorbance of 14150 at 412 nm.

3.3.6.3 Effects of Temperature on Allicin Thermal Stability

i. Prepare 6 g/150 mL garlic extract (GE) stock solution by dissolving 6 g of garlic powder in 150 mL of deionized water.
ii. In 4 separate boiling flasks, prepare 50 mL of 0.02 g/mL garlic extract (GE) reagent by mixing 25 mL of stock solution and 25 mL of deionized water.
iii. Before the experiment, extract 0.5 mL of reagent from the respective boiling flasks to test for allicin content.
iv. The extracted samples are added to 1.2 mL of 2 mM L-cysteine and allowed to sit at room temperature for 10 minutes respectively. Then, 3 mL of 50 mM

HEPES buffer and 1 mL of 5 mM DTNB is added into the respective sample mixtures.

v. The sample mixtures are allowed to sit for 2 minutes before transferring them into respective cuvettes. The samples' absorbance is measured at 412 nm using a UV-Vis spectrophotometer. The initial absorbance reading for all samples are recorded.

vi. Prepare 4 water baths at temperatures 30°C, 35°C, 40°C and 85°C.

vii. When all 4 water baths has met their set temperatures, the timer is set to 0 hours and the boiling flasks containing 0.02 g/mL garlic extract are immersed in the water bath.

viii. At every 1 hour interval, 0.5 mL of garlic extract is removed from each sample to test for allicin content. Testing procedures stated in steps (iv) and (v) are repeated for each test.

ix. The concentration of allicin is tested at hourly intervals for 5 hours.

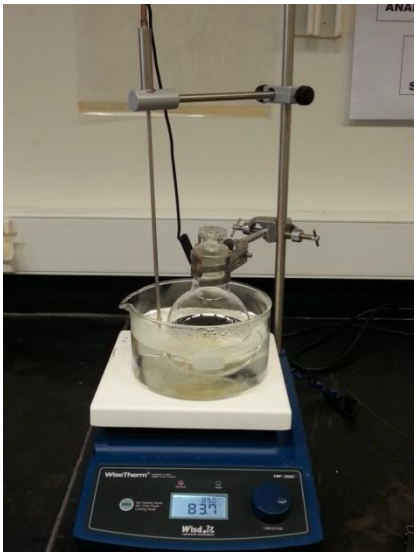


Figure 3.2 Equipment set up to test allicin thermal stability

CHAPTER 4

RESULTS & DISCUSSION

4.1 Experimental Results

4.1.1 Quantification of Allicin in Garlic Extract

Table 4.1 Allicin content (mM) at various garlic extract concentration (g/mL)

Sample	Concentration (g/mL)	Absorbance	L-Cysteine Remaining (mM)	Allicin Content (mM)
A	0.005	2.127	0.1503	0.6011
B	0.010	1.911	0.1351	0.6061
C	0.015	1.763	0.1246	0.6095
D	0.020	1.621	0.1146	0.6127
E	0.025	1.655	0.1170	0.6120

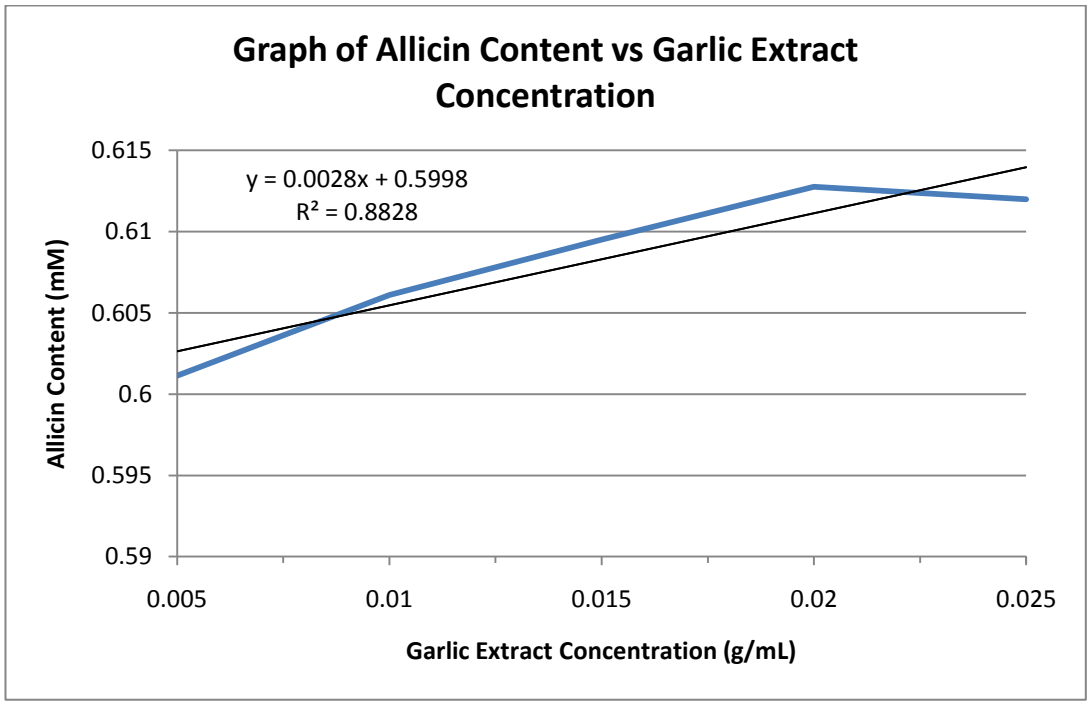


Figure 4.1 Graph of Allicin content versus Garlic Extract Concentration

4.1.2 Effects of pH on Allicin Formation

Table 4.2 Sample absorbance at various pH

pH	Absorbance
4	0.2204
5	0.2097
6	0.2016
7	0.2037
8	0.2030

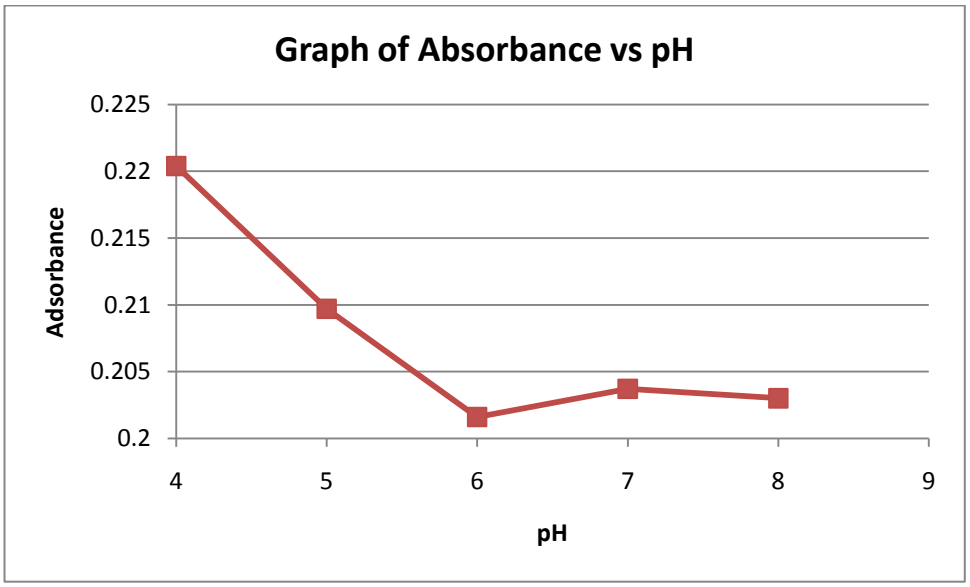


Figure 4.2 Graph of Absorbance versus pH

4.1.3 Effects of Temperature on Allicin Thermal Stability

Table 4.3 Hourly absorbance reading at various temperatures

	Absorbance at Different Temperatures			
Hours	30 ℃	35 ℃	40 ℃	85 ℃
0	1.618	1.618	1.618	1.618
1	1.619	1.643	1.696	2.075
2	1.619	1.662	1.752	2.137
3	1.623	1.672	1.773	2.142
4	1.626	1.707	1.838	2.145
5	1.624	1.711	1.957	2.147

Table 4.4 Hourly allicin content at various temperatures

	Allicin Content at Different Temperatures (mM)			
Hours	30 ℃	35 ℃	40 ℃	85 ℃
0	0.61284	0.61284	0.61284	0.61284
1	0.61281	0.61226	0.61105	0.60234
2	0.61281	0.61183	0.60976	0.60092
3	0.61272	0.61160	0.60928	0.60080
4	0.61265	0.61079	0.60778	0.60073
5	0.61270	0.61070	0.60505	0.60069

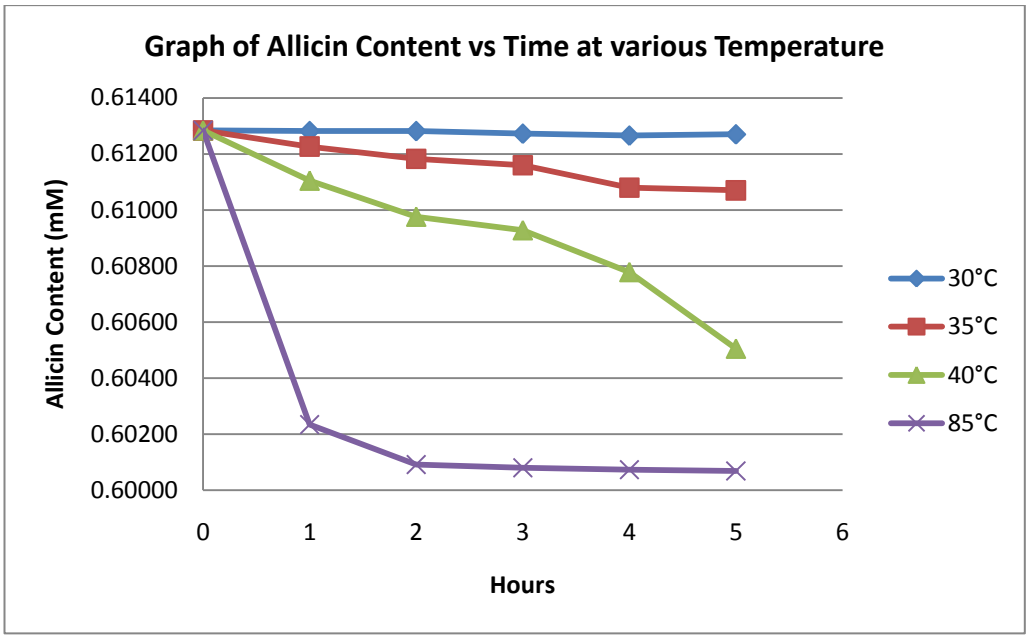


Figure 4.3 Graph of Allicin Content versus Time at Various Temperatures

4.2 Discussion

4.2.1 Quantification of Allicin in Garlic Extract

From the results obtained, it could be seen that allicin concentration increases with the increase in garlic extract concentration. 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. (Han et al., 1995). When the amount of NTB present is known, back calculation is done to find out the amount of allicin originally present in the sample. 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 based on the fact that allicin makes up 60 – 80% of thiosulfinates present in garlic extract (Sariri et al., 2002).

From the linear plot, a straight line formula is derived to be $Y = 0.0028X + 0.5998$, where Y is allicin content (mM) and X is garlic extract concentration (g/mL). This relationship is restricted to quantification of allicin at 30°C and it should only be used on freshly prepared garlic extract. It will provide future researchers with an estimate on amount of allicin present in garlic extract without the need to perform complicated quantification tests.

4.2.2 Effects of pH on Allicin at 30°C

The results show that the ideal pH for allicin stability is pH 6, which is also the control pH. It was also found that allicin tends to decompose more when subjected to acidic conditions compared to alkaline conditions. This finding contradicts results by Sariri et al. (2002), which stated that allicin amount was unaffected when subjected to acidic conditions. The result also contradicts findings by Lawson et al. (1992) which state that allicin is stable at the pH range of 4.5 – 5.0. However, both Lawson et al. (1992) and Sariri et al. (2002) did not mention the

conditions at which their experiment was carried out. It can be deduced that variation in experimental temperature could lead to different results. This is because allicin is highly unstable in nature. When acidic conditions are coupled with slight heating, it could increase the rate of decomposition of allicin.

Another possible explanation to allicin's low tolerance to acidic environment is the present of nucleophiles in acidic environment. The disulfide bond within its allicin is highly susceptible to nucleophilic attacks (Benavides et al., 2007 ;Robyn et al., 2013). When the S-allyl bond in the R-S(O)S-allyl compound is split, allicin decomposes

On the other hand, the results obtained from this experiment shows that all readings are close to zero. This is due the concentration of L-cysteine being just sufficient to indicate the presence of allicin in the sample. Hence, only trace amount of leftover L-cysteine was able to react with DTNB to form NTB and thus, give rise to the near zero absorbance reading. This problem could be avoided if a more concentrated L-cysteine solution is used or the 2.0 mM L-cysteine solution is used in greater volume.

4.2.3 Effects of Temperature on Allicin Thermal Stability

From the results, it could be seen that allicin decomposes at a faster rate when subjected to higher temperatures. We can also deduce that allicin and other thiosulfinates fully decomposes as temperature approaches 85°C as there is only negligible amount of allicin left 1 hour into the experiment at 85°C. This observation is different to that in Sariri et al.'s (2002) research where garlic extract was subjected to 80°C for 1 hour and boiling water for 30 minutes in two separate experiments. They reported that the concentration of allicin was not affected by these two extreme conditions. However, it should be noted that allicin is an organic compound and it should decompose when it is heated. Typically, a disulfide bond (S-S) has bond dissociation energy of 250 kJ/mol. Hence, this makes the disulfide bond present in allicin is 40% weaker than C – C and C – H bonds (Shin et al., 2014). The disulfide bond forms the weakest link in the allicin structure and thus, breaks easily when excessive heat is supplied.

4.3 Problems Encountered

4.3.1 Hot Plate Temperature Control

The hot plate is used extensively in all experiments to maintain constant temperature. At near ambient temperatures such as 30°C, 35°C and 40°C, the hot plate is not able to maintain the set temperature immediately and causes temperature to spike above the set temperature. High temperature leads to inaccuracy of data due to allicin and other thiosulfinates being decomposed.

Upon studying the instruction manual, it was found that the hot plate's temperature control is a proportional, integral and derivative (PID) controller. When a PID controller is used, the real value will oscillate around the set value for a period of time before the set value can be met.

Hence, at least one hour time has to be given to the hot plate to reach its set value before it could be used to maintain the temperature of samples.

4.3.2 Availability of Quartz Cuvettes

During spectrophotometric tests for the samples, samples have to be placed in quartz cuvettes. This is because 2-nitro-5-thiobenzoate (NTB) is spectrophotometrically active in the UV region (Han et. al, 1995) and quartz cuvettes are most suitable for absorbance reading at this region.

However, there is only 2 quartz cuvettes available for each experiment. One is used to carry the blank deionized water and the other is used to contain the sample. Due to this limitation, the sample cuvette has to be cleaned and dried after every reading to prevent contamination. This process is time consuming and it could be avoided if more cuvettes are available.

CHAPTER 5

CONCLUSION & RECOMMENDATIONS

In conclusion, this research project is a stepping stone to integrate allicin as urease inhibitor in urea fertilizers The results of this project has given us insight on how allicin behaves when subjected to conditions various pH and temperature. In the context of local Malaysian climate, allicin is suitable to be integrated into urea fertilizers as inhibitor because it is able to remain stable in slightly acidic (pH 6) conditions. Apart from that, the local temperature will not cause allicin to decompose rapidly. Allicin's rate of decomposition remained slow at 30°C. However, it should be noted that allicin must not be present in the manufacturing of urea fertilizers as the process operates under high temperature.

Future research work should further explore heat tolerance of allicin to a greater extent. The presence of allicin at 30°C should be monitored up to the point that it fully decomposes. Besides that, allicin concentration should also be monitored with time at the pH range of 5 to 6. These conditions suit the local climate and ground conditions. Measuring allicin stability in realistic soil conditions can also help uncover more of its behaviour and help realize slower leaching urea fertilizers in the near future.

Apart from that, this research also involves the usage of food as an additive in the agricultural industry. This move contradicts the shortage of food supply faced by poverty stricken parts of the world. Besides, garlic is an expensive commodity used is the culinary industry. Hence, in order to boost the economic viability of allicn as a bio-inhibitor, it is suggested to source for an alternative allicin source other than garlic.

.

REFERENCES

1. Juszkiewicz, A., Zaborska, A., Laptaś, A. & Olech, Z. (2003). A study of the inhibition of jack bean urease by garlic extract. *Food Chemistry*, 2003, Vol. 85, pp. 553 – 558.
2. Qi, R., Wang, Z. (2003). Pharmacological effects of garlic extract. *Trends Pharmacol. Sci.*, 2003, Vol. 24, pp. 62 - 63.
3. Olech, Z. & Zaborska, W. (2012). A spectrophotometric assay for total garlic thiosulfinates content. *Pol. J. Food Nutr. Sci.*, 2012, Vol. 62(1), pp. 23 – 29.
4. Kyung, K.H. & Lee, Y.C. (2001). Antimicrobial activities of sulphur compounds derived from S-alk(en)yl-L-cysteine sulfoxides in Allium and Brassica. *Food Rev. Int.*, 2001, Vol. 17, pp. 183 – 198.
5. Cavallito, C.J. & Bailey, J.H. (1944). Allicin, the antibacterial principle of Allium sativum. Isolation, physical properties and antimicrobial action. *J. Am. Chem. Soc.*, 1944, Vol. 66, pp. 1950 – 1951.
6. Rosen, R.T., Hiserodt, R.D., Fukuda, E.K., Ruiz, R.J., Zhou, Z., Lech, J., Rosen, S.L. & Hartman, T.G. (2001). Determination of allicin, S-allylcysteine and volatile metabolites of garlic in breath, plasma or simulated gastric fluids. *J. Nutr.*, 2001, Vol. 131, pp. 968S – 971S.
7. Arnault, I., Christidès, J.P., Mandon, N., Haffner, T., Kahane, R. and Auger, J. (2003). High-performance ion pair chromatography method for simultaneous analysis of alliin, deoxyalliin, allicin and dipeptide precursors in garlic products using multiple mass spectrometry and UV detection. *J. Chromatogr.A.*,2003, Vol. 991, pp. 69 – 75.
8. Keusgen, M., Jünger, M., Krest, I. & Schönning, M.J. (2003). Development of a biosensor specific for cysteine sulphoxides. *Biosensors & Bioelectronics*, 2003, Vol. 469, pp. 155 – 164.
9. Miron, T., Shin, I., Feigenblat, G. Weiner, L., Mirelman, D., Wilchek, M. & Rabinkov, A. (2002). A spectophotometric assay for allicin, alliin and alliinase (Alliin lyase) with chromogenic thiol: reaction of 4-mercaptopyridine with thiosulfinates. *Anal. Biochem.*, 2002, Vol. 307, pp. 76 – 83.

10. Han, J., Lawson, L., Han, G. & Han, P. (1995). Spectrophotometric method for quantitative determination of allicin and total garlic thiosulfinates. *Anal. Biochem.*, 1995, Vol, 225, pp. 157 – 160.
11. Yoo, K.S., Pike, L.M. & Hamilton, B.K. (2001). Determination of background pyruvic acid concentrations in onions, Allium species and other vegetables. *Sci. Hort.*, 2001, Vol. 89, pp. 249 – 256.
12. Bocchini, P., Andalo, C., Pozzi, R., Galletti, G.C. & Antonelli, A. (2001). Determination of diallyl thiosulfinate (allicin) in garlic (Allium sativum L.) by high-performance liquid chromatography with a post-column photochemical reactor. *Analytica Chemica Acta*, 2001, Vol. 441, pp. 37-43.
13. Kawaguchi, K. & Kyuma, K (1974). Paddy soils in tropical asia. *Southeast Asian Studies*, 1974, Vol. 12(1), pp. 3 – 24.
14. Yu T. R. (1991). Characteristics of soil acidity of paddy soils in relation to rice growth. *Developments in Plant and Soil Sciences*, 1991, Vol. 45, pp. 107 – 112.
15. Lawson, L.D. & Hughes, B.G. (1991). Characterization of the formation of allicin and other thiosulfinates from garlic. *Planta Med.*, 1991, Vol. 58(4), pp. 345 – 350.
16. Sariri, R., Aliakbar, A., Mesdarghi, G. B., & Rezazadeh, A. (2002). Spectrophotometric assay of allicin in Iranian garlic products. Asian Journal of Chemistry, 2002, Vol. 14(3-4), pp. 1197 – 1202.
17. Benavides, G.A., Squadrito, G.L., Mills, R.W., Patel, H.D, Isbell, T.S., Patel, R.P., … & Kraus, D.W. (2007). Hydrogen sulphide mediates the vasoactivity of garlic. *Proc Nati Acad Sci U.S.A.*, 2007, Vol. 104(48), pp. 17977 – 17982.
18. Robyn, J., Rasschaert, G., Hermans, D., Pasmans, F. & Heyndrickx (2013). Is allicin able to reduce Campylobacter jejuni colonization in broilers when added to drinking water? *Poultry Science*, 2013, Vol. 92(5), pp. 1408 – 1418.
19. Shin, J. & Paek, K. (2014). The facile synthesis of a carceplex with four disulfide bridges. *Bull. Korean Chem Soc*., 2014, Vol. 35(7), pp. 2205 – 2206.