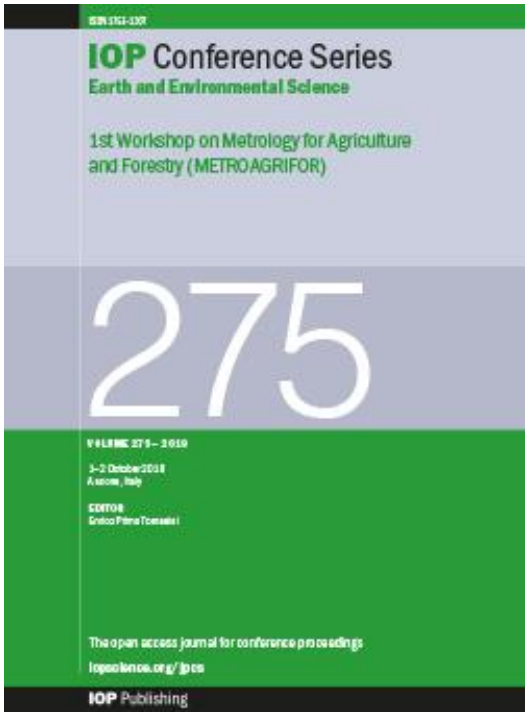
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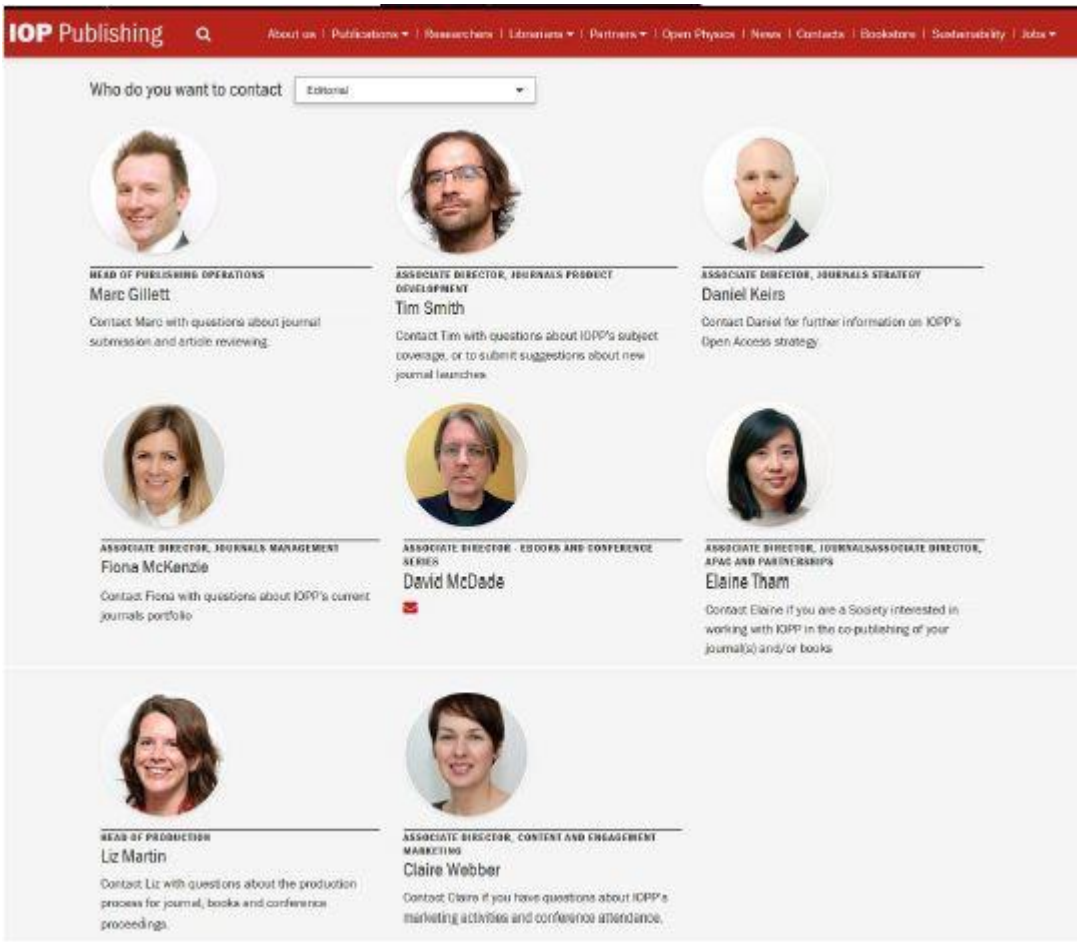
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Allicin Chemical Stability Test in the Phytosome of Garlic Extract *(Allium sativum* L)

Allicin Chemical Stability Test in the Phytosome of Garlic Extract *(Allium sativum* L)

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Abstract. Allicin contained in garlic extract is unstable at high temperatures. Meanwhile, this compound has the potential to be widely used as an antidiabetic. One way to optimize the use of allicin is by using a phytosome delivery system that can protect allicin so that it does not degradation easily. The purpose of this study was to determine the storage temperature which indicates the slow rate of degradation of allicin in the phytosomes of garlic extract (PGE). PGE were stored for 8 weeks at 4^{o}C, 25^{o}C and 40^{o}C. Allicin content were measured every 2 weeks using a UV-Vis spectrophotometer. The results of the degradation rate of allicin in PGE at temperatures of 4^{0}C; 25^{0}C; 40^{0}C were 0.0191; 0.0185; 0.0212, respectively. Based on these results, it can be concluded that the storage temperature of 25^{0}C showed the degradation rate of allicin in the PGE is slower than the other storage temperatures.

1. Introduction

The use of plants as medicinal materials has become the culture of almost every country in the world. One of the plants that has medicinal properties is garlic (Allium sativum L) which contains allicin (diallyl thiosulfonate or diallyl disulfide) [1]. Previous research showed that giving garlic methanol extract at a concentration of 400 mg / kgBW has the effect of reducing blood glucose levels in mice [2]. The problem of using natural ingredients as an active ingredient in a dosage form is the low stability because the extract is easily broken down. This problem can be overcome by formulating these natural ingredients into a drug delivery system. One of the delivery systems that can be used is phytosomes.

Phytosome is a technology developed from manufacturing drugs and nutraceuticals. Phytosomes can entrapped natural materials, most of which are hydrophilic so that they can be used to increase bioavailability and penetration [3]. Phytosomes compared to conventional herbal formulations can increase the efficacy of the therapeutic effect due to increased penetration by phosphatidylcholine so that the extract can better penetrate

the bilayer lipid membrane, besides that the possibility of the extract contained in the phytosome to break down is also smaller [4]. Garlic extract (Allium sativum L) can be made in a phytosome system using lecithin as a phospholipid bond-forming so it can increase its stability and absorption [5]. Phospholipids that are often used in the manufacture of phytosomes are phosphatidylcholine. Phosphatidylcholine is prone to hydrolysis in the aqueous system which can cause chemical instability of the vesicle system such as phytosomes which can further affect the distribution of particle size, surface charge value, permeability, phase, and pH [6]. To ensure that the phytosome protects allicin, it is necessary to test chemical stability by determining the rate of the degradation reaction of allicin carried out at temperatures of 4^0C, 25^0C and 40^0C.

Based on this explanation, the purpose of this study was to determine the storage temperature which indicated the slow rate of the reaction of allicin degradation in the phytosome of garlic extract (PGE).

2. Method

Materials: Spectrophotometer UV-Vis 1601 (SHIMADZU), GCMC-QP2010 (ULTRA-SHIMADZU), particle size analyzer (DELSA MAX), transmission electron microscopy (JEOL JEM-1010), pH meter (LaMotte), oven (MEMMERT), karl fischer (METROHM) and refrigerator (LG). Methanol extract of garlic (Allium sativum L) and soy lechytin (LANSIDA), S-Allyl 2-propane-1-sulfinothioate (SIGMA ALDRICH), absolute ethanol, dichloromethane (Pro analysis), potassium dihydrogenphosphate, sodium hydroxide and hydrogen disodium phosphate (MERCK) and aquadest.

Characteristics of garlic extract: Organoleptic was carried out after leaving the viscous extract for 24 hours which included observation of the form, color and odor at room temperature. The form is seen from the extract that is able to flow in a container. Color seen against a white paper background accompanied by lighting. The aroma is smelled by wiping it over the surface of the extract. Determination of water content using 0.05 grams of extract and determined by the Karl Fischer method.

Qualitative test using 100 mg of extract dissolved into 1mL of hexane. Then make allicin calibration standards by dissolving allicin in hexane with dilution variations of 0.125 ppm, 0.25 ppm, 0.5 ppm, 1 ppm and 2 ppm. The sample is injected into the septum by means of a split injection. The type of column used is HP-ULTRA2 agilent. Column oven temperature 800C, injection temperature 2300C for 25 minutes with helium gas mobile phase and the detector using MS (Mass Spectrophotometer).

Yield calculation [7] : $\text{Yield} = \frac{\text{Extract weights}}{\text{Garlic bulbs weights}} \text{ x } 100\%$

Determination of total ash content [8] by using 2g of the extract carefully weighed, and put in a silicate crucible that has been incandescent and tared, then slowly incandescent until the charcoal runs out, cool and weigh. Remove the filter paper along with the rest of the filter in the same crucible. Put the filtrate into the crucible, steam and incandescent until the weight remains. The total ash content is calculated against the weight of the test material expressed in% w / w.

Determination of acid insoluble ash content [8] by boiling the ash obtained on the assay of the total ash content with 25 mL dilute hydrochloric acid for 5 minutes. Collect the parts that are insoluble in acid, filter through an ash-free filter paper, wash with hot water, incandescent in a crucible until a fixed weight. The ash content that is insoluble in acid is calculated against the weight of the test material, expressed in% w / w.

PGE [9]: PGE was prepared by complexing phospholipids and methanol extract of garlic (Allium sativum L) using a thin layer hydration method. Soy phosphatidylcholine was dissolved with dichloromethane (DCM) while the garlic extract was dissolved with 96% ethanol, then put in a round bottom flask. Diclorometan is evaporated using a rotary evaporator at 30^0C with a speed of 125 rpm, then vacuumed until a thin layer is evenly distributed. The layer was stored in the refrigerator for up to 24 hours at 7^0C. The thin layer is hydrated with a phosphate buffer solution pH 5.5. After that, sonication was carried out for 60 minutes.

PGE evaluation: Organoleptic was carried out after leaving the PGE for 24 hours which included observation of the form, color and odor at room temperature. The form is seen from the extract that is able to flow in a container. Color seen against a white paper background accompanied by lighting. The aroma is smelled by wiping it over the surface of the extract.

Density test [10] by using a pycnometer. This is done by setting the weight of the empty pycnometer (W0) and the weight of the pycnometer filled with water at a temperature of 25^0C (W1). Enter the sample into the pycnometer to remove the excess and weigh (W2). Density of the sample is the result obtained by dividing the sample weight by the weight of water, in a pycnometer at 25^0C. Density is calculated by: $\rho = \frac{W2-W0}{W1-W0}$

Determination of particle size (PS), polydispersity index (PI), zetta potential (ZP) [11] by using particle size analyzer (PSA). The sample was diluted with aqua pro injection (1 mL of sample mixed with 9 mL of aqua pro injection). The solution is inserted into the flow cell which is then inserted into the instrument. The device is turned on and the DLS & PALS (Stimultaneous) menu is selected. The instrument will measure the sample for 3 minutes, then the PS, PI, and ZP of the PGE vesicles will be measured.

Determination of entrapment efficiency (EE) by using 1mL of PGE was centrifuged to separate the active substance which was not entrapped in PGE at a speed of 10,000 rpm for 60 minutes. The supernatant was taken to measure the content of allicin that was not entrapped in PGE's vesicles. Then the volume is sufficient with a solvent mixture of phosphate buffer pH 6.8 and ethanol 95% to 10 mL, a 0.5 mL pipette then put into a 10 ml measuring flask, the volume is sufficient using a mixed solvent to mark the limit. The solution obtained was measured its absorbance using a spectrophotometer at a maximum wavelength of 214.4 nm. The levels obtained were free allicin levels (FAL). Then to the precipitate was added 0.5 mL of DCM, vortexed for 1 minute, and put into a 10 mL measuring flask and sufficiently volume up to the limit mark. The 0.5 mL pipette is then put into a 10 ml measuring flask, the volume is sufficient using a mixed solvent to the limit mark. The solution obtained was measured for its absorbance using a spectrophotometer. The level obtained is entrapped allicin level (EAL). The percentage of allicin absorbed is determined directly using the formula: $\%\, EE = \frac{EAL}{EAL + FAL}$ X100%

PGE morphology was determined using TEM (transmission electron microscopy). A sample of 1 mL was dissolved in 1 mL of aqua pro injection then vortexed for 1 minute. From this solution, pipette as much as 5 µl and allowed to dry, then dropping 0.5% uranyl acetate (UA). After that the sample is observed under a microscope with various magnifications in accordance with the expected image results.

Chemical stability testing: to see the decrease in the degradation of allicin from PGE using 3 different temperatures, i.e 4^0C, 25^0C and 40^0C. The test was carried out for 8 weeks. The test procedure is the same as the entrapment efficiency. The allicin level obtained was entered into the equation of the zero and first order allicin degradation model

The evaluation which was analyzed statistically was the degradation rate. The data obtained were normally distributed and homogeneous, then analyzed using the 1-way ANOVA test, followed by the Tukey HSD test.

3. Results and Discussion

The results of the characteristics of the garlic extract can be seen in **TABLE 1**. Determination of water content aims to provide a maximum limit of the amount of compounds lost in the drying process and to meet the water standard in dried simplicia with a requirement of not more than 12% [8]. High water content or more than 12% can be a medium for mold and fungus growth so that it can reduce the quality of simplicia. The low water content is expected to minimize the possibility of simplicia being able to grow molds and fungi so that the extract quality remains good and can be stored for a long time [12]. The water content of the garlic extract obtained was 1.21%, indicating that the water content of the garlic extract was in accordance with the requirements of the Indonesian herbal pharmacopoeia, which was less than 12%.

Determination of the total ash content aims to determine the internal and external mineral content originating from the initial process until the extract is formed. In the process of testing the total ash content, the extract is heated until the organic compounds and their derivatives are digested and evaporated until only the mineral and inorganic elements are left. The total ash content of garlic powder obtained was 3.34%, indicating that the total ash content of the extract was not in accordance with the requirements, i.e not more than 2.7% [8]. A high total ash content indicates that the substance contains high metal content.

Determination of acid insoluble ash content aims to determine the residual acid insoluble ash in the form of heavy metals. Acid insoluble ash content reflects the presence of mineral or metal contamination that is not

acid soluble in a product. Ash content is insoluble in acid usually contains silicates derived from soil or sand. The amount of dirt, soil, clay and metal elements Ag, Pb and Hg [12]. The acid insoluble ash content of garlic extract obtained was 0.15% indicating that the acid insoluble ash content of the extract was in accordance with the requirements of the Indonesian herbal pharmacopoeia, which is less than 1. Low acid insoluble ash content indicates that the substance contains low internal minerals.

Table 1. The characteristics of the garlic extract

No	Parameter	Result
1.	Form	Viscous extract
2.	Colour	Brown
3.	Odor	Aromatic pungent
4.	Taste	Bitter
5.	Water content	1,21 %
6.	Total ash content	3,43 %
7.	Acid insoluble ash content	0,15 %
9.	Yield	15,97 %

The qualitative test results of allicin using GC-MS to determine the content of allicin compounds (S-Allyl-2-Propene-1-Sulfinothioate) based on the total chromatogram ions, especially for volatile compounds. Analysis of the results of the GC-MS chromatogram (**FIGURE 1**) shows that there is a similarity at the peak between garlic extract and allicin standard which is marked by one marker compound at the retention time of 7,722, namely the allicin compound (S-Allyl-2-Propene-1-Sulfinothioate) which is a compound of the organosulfur group.

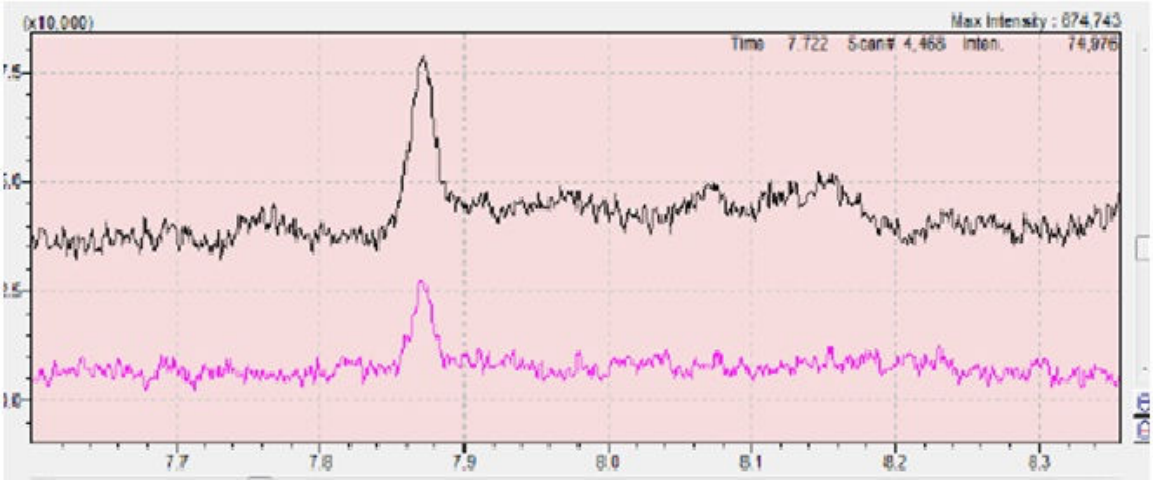

Figure 1. GC-MS Chromatography Results: Black chromatogram (sample), pink chromatogram (Allicin standard)

Phytosomes have vesicles that are nanometer in size, with a particle size of 1-300 nm [13]. From the measurement results, it can be seen that the PGE particle size obtained is 270.0 nm (**TABLE 2**), so that the PGE produced is still included in the nanoparticle preparation. The excess of small drug particles can make it easier for drug particles to penetrate the membrane [14]. Polydispersity refers to the degree of homogeneity of the particle size. The smaller the PI value then the more homogeneous the nanoparticle dispersion. If the PI value is less than 0.15 the particles are considered homogeneous or monodisperse and if the PI value is greater than 0.35 the particles can be considered non-homogeneous or polydisperse [15]. The result of the PGE

polydispersity index value is 0.571 so that the phytosome is relatively non-homogeneous. Zeta potential is a measure of the repulsive force between particles. ZP is useful for measuring the charge on the surface of particles. The positive and negative values of ZP indicate the outer charge of the particles of the ionized phospholipid to form OH- when dispersed in aquadest. The value on the surface charge of the particles is useful for describing colloid stability. Nanoparticles with a ZP value close to ± 30 mV are proven to be stable, the stability of the preparation is poor if the zeta potential value is small than 30 and a preparation is said to be stable if the ZP value is greater than 30 [16]. From the data obtained, the ZP value is -32.55 mV, meaning that PGE with a ZP value greater than 30 mV has good stability.

The density was measured to determine the characteristics of the phytosome, the result obtained from the measurement was 1.0208 g / mL. Substances that have a density less than 1.00 g / mL are lighter than water. Substances having a density greater than 1.00 are heavier than water. From the results obtained, PGE has a density slightly greater than water. The determination of allicin EE in PGE was carried out to determine the percentage of allicin entrapped in the phytosome. The evaluation results showed 64.8798%. The result of low entrapment efficiency can be influenced by 2 factors. The first factor is the low allicin content in the garlic extract, this is influenced by the temperature of the solvent evaporation during the extraction process to obtain a viscous extract. The second factor is the conditions for produce phytosomes, including speed, temperature, duration of thin layer formation and during hydration, as well as insufficient lecithin concentration to bind the active substance into the phytosome.

Table 2. Result of PGE evaluation

No	Parameter	Result
1.	Organoleptic	
	- color	Brown
	- form	Liquid
	- aroma	Specific
	- taste	Bitter
2.	Density	1,0051 g/mL
3.	ZP	-32,55 mV
4.	PI	0,571
5.	PS	270 nm
6.	EE	64,8798 %

The Transmission Electron Microscope was used to see the vesicle shape and surface morphology of the PGE vesicle. TEM tomography has a greater resolution so that the resulting image can be enlarged. This evaluation provides information on the size of the PGE vesicle at 200nm. TEM is a microscopy technique with the principle of emitting a beam of electrons, interacting with and hitting thin specimens. From the interaction of the transmitted electrons an image is produced which can then be enlarged, focused on an image device such as a film photographic layer. TEM can see morphology, structure to the atomic level. The morphological data obtained with a magnification of 30,000x showed that some vesicles were round but not symmetrical, and there were also regular rounds with varying shapes. The results of the TEM evaluation can be seen in **FIGURE 2**.

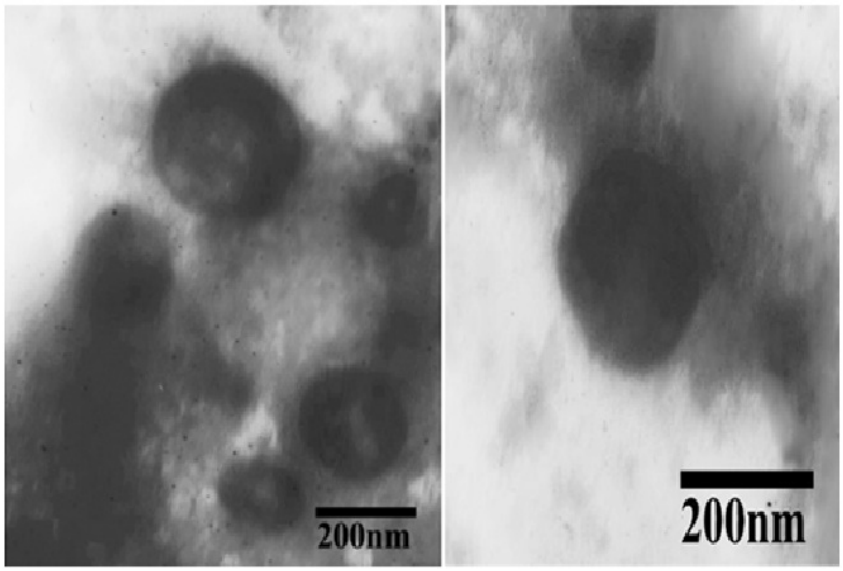

Figure 2. Result of TEM

With the length of storage, there was a decrease in allicin levels in PGE. The reaction will proceed faster if energized from the outside by increasing the temperature. In general, the rate of reaction is doubled for every 10^0C increase in temperature. The degradation reaction is faster due to an increase in temperature [17]. The reaction order kinetics of allicin degradation in PGE follows a zero order, where the magnitude of the reaction rate is not influenced by the amount of concentration. The reaction rate of allicin degradation in PGE can be seen in **FIGURE 3**. Meanwhile, the degradation rate of allicin in PGE is at a temperature of 4^0C> 25^0C <40^0C. At 4^0C, the degradation rate of allicin is greater than at 25^0C, this happens because of the nature of phosphatidylcholine which will experience separation if stored at temperatures below 10^0C [18]. While the degradation rate of 40^0C is greater than 25^0C, this is in accordance with the literature that the reaction rate increases to two times every 10^0C temperature increase [17]. Previous research has also shown that the higher the storage temperature, the greater the degradation of phosphatidylcholine, the increase in storage temperature will lower the pH of the system which triggers an acid-base catalyst reaction, the addition of buffer can minimize the decrease in system pH thereby slowing down the degradation of phosphatidylcholine [6]. In addition to temperature, the length of storage time can increase the optical density so that it can decrease the physical stability of the vesicle system [19].

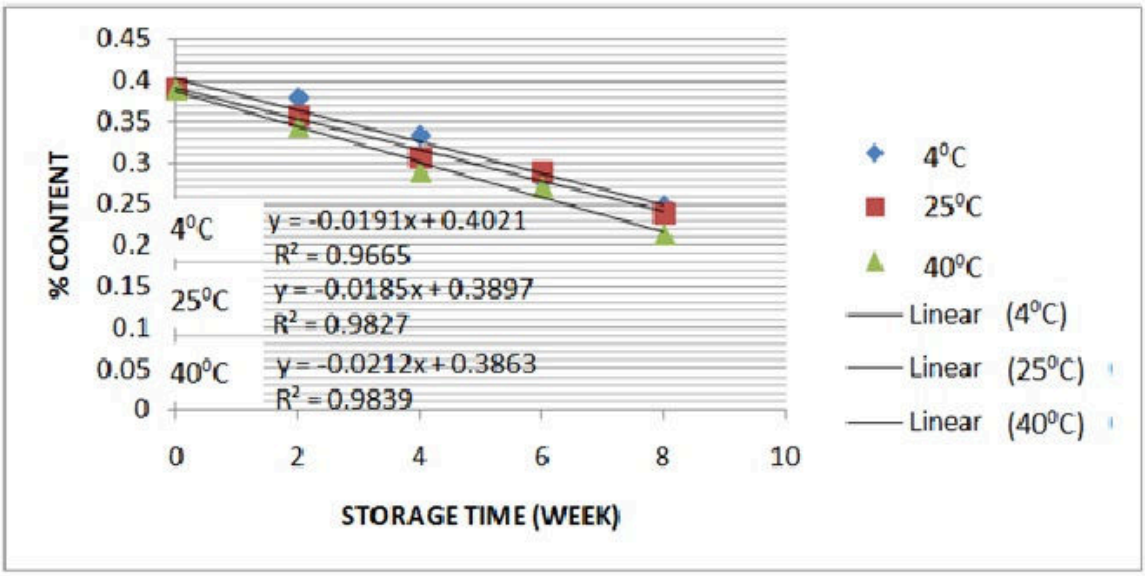

Figure 3. Degradation rate of allicin

Based on statistical analysis, evaluation of the degradation rate of allicin in PGE was carried out using one way ANNOVA. The results of the test obtained asymp.Sig. (2-tailed) 0.047. The significance value obtained was <0.05, which means that there was a significant difference in the degradation rate of garlic phytosomes to storage temperature. The Tukey HSD result shows the Sig. 0.047 <0.05, which indicates that there is a significant difference between the degradation rate at 25^{0}C and 40^{0}C.

4. Conclusion

Based on the results of the research that has been done, it can be concluded that the reaction rate of the degradation of allicin in the form of PGE is slower at 250C compared to other temperatures.

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