Research Article

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Influence of pH, concentration and light on stability of allicin in garlic (*Allium sativum* L.) aqueous extract as measured by UPLC

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Abstract

BACKGROUND: Garlic is one of the most important bulb vegetables and is mainly used as a spice or flavoring agent for foods. It is also cultivated for its medicinal properties, attributable to sulfur compounds, of which allicin is the most important. However, the stability of allicin in garlic extract is not well understood. In this study, using UPLC, the stability of allicin extracted in water from garlic was evaluated in phosphate buffer at different temperatures under light and dark conditions.

RESULTS: At room temperature, allicin in aqueous extract was most stable at pH 5–6 but degraded quickly at lower or higher pH. It began to degrade within 0.5 h and was not detectable after 2 h when the pH was higher than 11 or lower than 1.5. It degraded quickly when the temperature was higher than 40 °C and especially higher than 70 °C. At room temperature, allicin in water could be stored for 5 days without obvious degradation. Higher concentrations of allicin in solution were somewhat more stable than low concentrations.

CONCLUSION: Allicin extract was sensitive to pH and temperature of storage but not to light. Higher-concentration allicin solution was more stable.
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Keywords: garlic; allicin; ultra-performance liquid chromatography; stability

INTRODUCTION

Garlic (*Allium sativum* L.) is one of the most important bulb vegetables and is mainly used as a spice or flavoring agent for foods. It is also cultivated for its medicinal properties, attributable to sulfur compounds such as allicin.[1,2] Allicin was discovered by Chester John Cavallito and John Hays Bailey in 1944[3] and then other scientists.[4,5] Allicin is present throughout the garlic plant, with the highest levels being found in extracts from whole green garlic, followed by shoot and leaf extracts.[6] However, the root bulb is considered to be the most important source of allicin. Crushing of garlic produces high but variable amounts of allicin ranging from 16.1 to 130.3 g kg^{-1} dry weight.[7]

Allicin has been found to have numerous antimicrobial properties. The inhibitory activity of old extract of garlic is similar to that of pure allicin, and fresh extract of garlic inhibits pneumolysin hemolytic activity at lower concentrations.[8] Many previous studies on animals, plants and humans have described the antifungal and antibacterial activities of allicin.[9–15] Other research has shown that allicin from garlic might be effective in some areas of clinical practice.[16–18]

In recent years a number of reports have appeared showing bacteriostatic activity of garlic aqueous extracts.[19–21] Aqueous extracts of garlic are widely used in daily life, especially in China. For example, aqueous extract of garlic is used in flour for dumplings, a very popular food in China. Some people like to make *kimchi* using garlic with vinegar, which is thought to retain the allicin in garlic for a longer time because of the lower pH of vinegar. In some areas, farmers use aqueous extract of garlic as an antiseptic by spraying it on forage to feed animals.[22] However, these positive properties are reduced because of the unstable character of allicin. The stability of allicin is affected by its environment. Once it is generated, allicin readily changes into other compounds when it is exposed to adverse temperatures or solutions. Thus cooking, aging or otherwise processing garlic causes allicin to be decomposed into other compounds.[23,24] It will degrade when subjected to high temperatures or high-pH solutions.[25,26] Previous studies based on biological and chemical assays demonstrated that allicin extracts are unstable and degrade quickly at high temperature.[27,28] Although the stability of allicin in aqueous extract at different temperatures from −80 to 42 °C has been studied,[6,25,27] the half-life of pure allicin and natural allicin in aqueous extract has not been reported consistently. The half-life of allicin in water at 23 °C reported by Fujisawa *et al.*[27] was four times shorter than that reported by Lawson and Gardner.[26] Besides, the stability of allicin in aqueous extract at higher temperatures has not been well studied. Moreover, the

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stability of allicin is affected strongly by the pH of the solution.[29] Owing to its instability in the acid environment (pH 1.2–2) of gastric fluid, allicin might degrade when passing through the stomach before it reaches blood and other tissues, so it is doubtful whether it contributes to any antithrombotic or blood-thinning actions in the body.[30] Thus it is of scientific interest to understand which pH is most suitable for allicin. Whether other factors such as concentration and light will affect the stability of allicin has not been reported yet.

To quantify allicin by typical high-performance liquid chromatography (HPLC), it takes a run time of about 12 min.[6] In previous work we developed a stable, quick ultra-performance liquid chromatography (UPLC) method that can detect allicin efficiently in a much shorter time of about 3 min.[2] This could help us to understand the stability of allicin in aqueous extracts under extreme environments, i.e. at high temperatures (>70 °C) or in highly acid (pH >10) or alkaline (pH <2) solutions, where it will degrade very quickly within a few minutes and thus typical HPLC will be unsuitable. In this study, using the UPLC method, the stability of allicin in aqueous extract at different concentrations, temperatures, pH values and light conditions was investigated.

MATERIALS AND METHODS

Reagents

Allicin standard solution (1100 mg L^{-1}) was purchased from Chromadex (Irvine, CA, USA). Acetonitrile, methanol (HPLC grade) and other reagents were obtained from Merck (Darmstadt, Germany), except for formic acid, which was purchased from DIMA Technology (San Dimas, CA, USA). Monosodium phosphate and disodium phosphate were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Water was purified through a Milli-Q water purification system (Bedford, MA, USA).

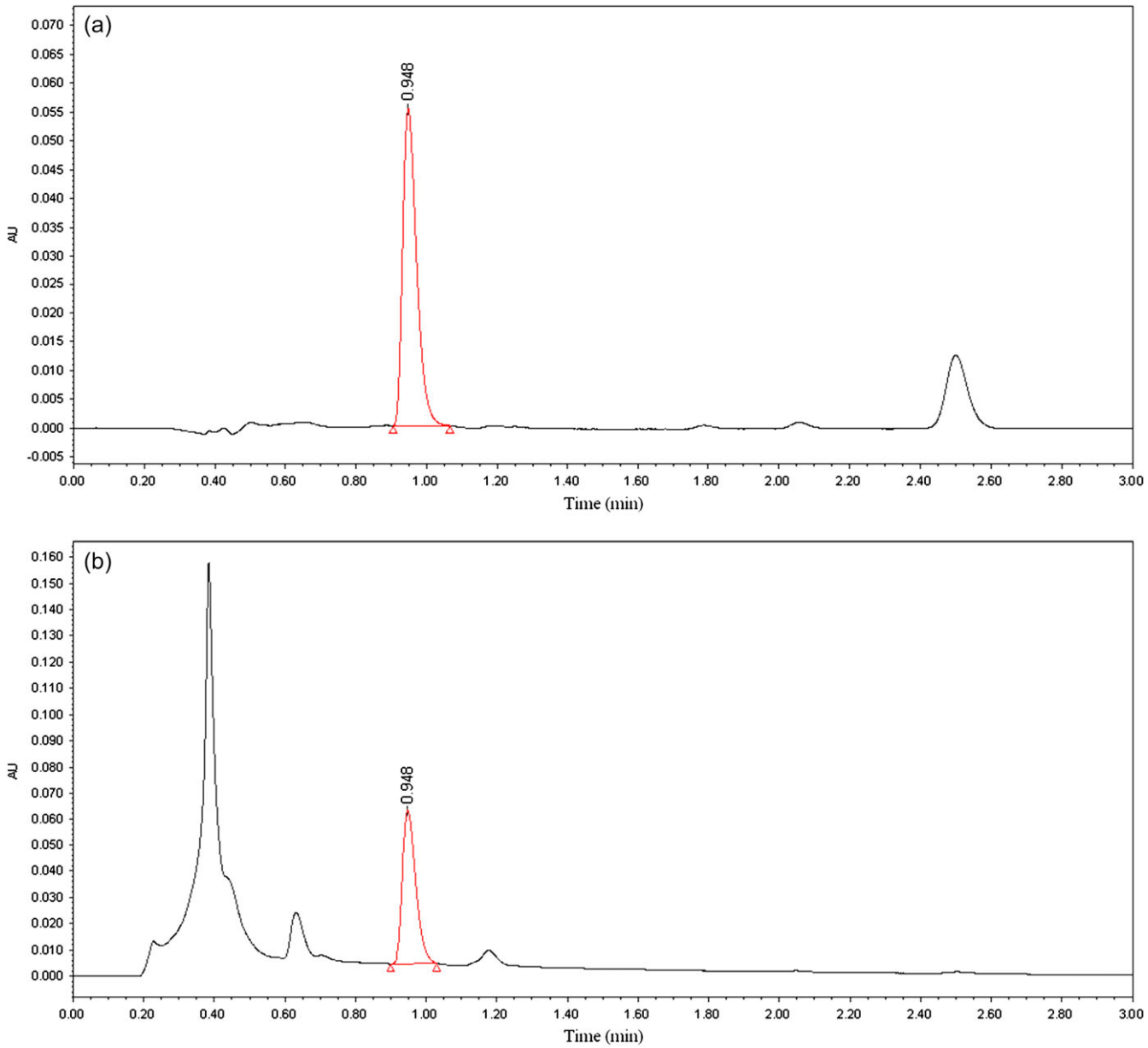

Figure 1. Chromatograms of (a) allicin standard and (b) sample. Peaks are at 0.95 min retention time.

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Preparation of garlic aqueous extract

The garlic variety 'De Zhou Hong Pi' (National Germplasm Repository for Vegetatively Propagated Vegetables, Beijing, China) was used in all experiments. Allicin was extracted from lyophilized garlic cloves according to the method of Wang *et al.*[2] Garlic cloves were peeled to remove the dry protective layers, kept at −20 °C for 3–4 h and chopped into small slices. The garlic slices were arranged in a thin layer, held at −80 °C for 3–7 h until frozen to dryness, ground to powder and passed through a 400-mesh sieve. Two replicates of 400 mg of garlic powder were accurately weighed into individual 50 mL centrifuge tubes, and 15 mL of cold water was pipetted into each tube. The tubes were immediately capped and shaken vigorously for 15 s, then another 15 mL of cold water was added to each tube and mixed for 30 s. The tubes were centrifuged at 8000 × *g* for 10 min at 4 °C. Each supernatant was filtered through a 0.45 μm Millipore filter into a vial before injection. The original mix extracts of the two tubes were used for the following experiments. Firstly, the allicin concentration of the original mix extracts was evaluated by the UPLC method described below. Secondly, the original mix extracts were diluted with purified water to concentrations of 9.03, 6.02 and 3.01 mg L^{-1} to assess the effects of temperature and light on the stability of allicin. Thirdly, the original mix extracts were diluted with monosodium phosphate and disodium phosphate to concentrations of 9.03, 6.02 and 3.01 mg L^{-1} to assess the effects of pH on the stability of allicin.

Quantitative analysis of allicin

The content of allicin was determined by UPLC as described by Wang *et al.*[2] UPLC analysis was performed with a Waters Acquity UPLC system controlled by Waters Empower software. Samples were evaluated on a UPLC BEH C18 column (2.1 mm × 50 mm, 1.7 μm particle size) with a mobile phase consisting of methanol/water (50:50 v/v) at a constant flow rate of 0.2 mL min^{-1}. The run time was set to 3 min. The column was kept at 28 °C and the sample chamber at 4 °C. Allicin was detected at a UV wavelength of 254 nm.

A stock solution of standard allicin was diluted with 1 mL L^{-1} formic acid to obtain a series of concentrations (510, 255, 170, 127.5, 51, 10.2 and 20.4 mg L^{-1}). A linear equation was constructed between peak areas and allicin concentrations. Concentrations of allicin in aqueous extracts were calculated from this equation.

pH effects on allicin stability

A series of phosphate buffers with pH ranging from 1.5 to 11 was prepared with monosodium phosphate and disodium phosphate. As mentioned above, the original aqueous extracts were diluted to concentrations of 9.03, 6.02 and 3.01 mg L^{-1}. Samples (three replicates for each treatment) were kept at a temperature of 25 °C and the stability of allicin was evaluated at 0, 0.5, 1, 3, 5, 7, 9, 120, 360, 480 and 720 h. The stability of allicin was assessed using UPLC and quantitatively determined as described above. The residual ratio of allicin in solution was calculated as follows:

$$\text{residual ratio } (\%) = [(\text{initial concentration} - \text{detected concentration}) / \text{initial concentration}] \times 100$$

The allicin degradation fitted curve and half-life were calculated in R software.[27]

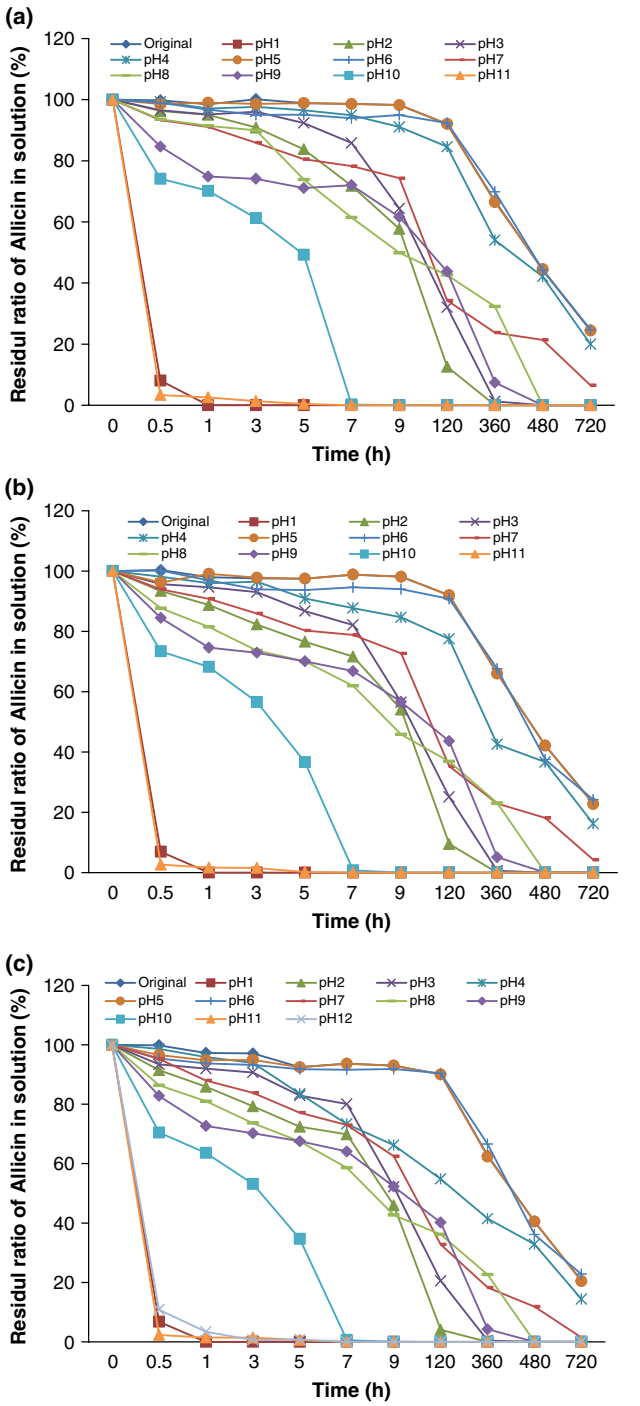

Figure 2. Stability of allicin in aqueous extract as a function of pH at (a) high (9.03 mg L^{-1}), (b) medium (6.02 mg L^{-1}) and (c) low (3.01 mg L^{-1}) concentrations. Data are mean of three replicates. Error bars denote ± SE.

Temperature effects on allicin stability

The stability of allicin at various storage temperatures was evaluated at concentrations 9.03, 6.02 and 3.01 mg L^{-1}. Samples (three replicates for each treatment) were stored in the dark at −20, 4, 25, 40, 55, 70 and 80 °C and the stability of allicin in aqueous extracts was evaluated after 0.5, 1, 1.5, 2, 3, 5, 7, 9, 120, 360, 480 and 720 h. For −20 °C, replicate samples were aliquoted and stored in injection tubes, which were allowed to thaw for 20 min before evaluation. For 4 and 25 °C, samples were placed in incubators.

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Table 1. Half-life of allicin as a function of pH, temperature (*T*, °C), concentration and exposure to light

Treatment	Conc.	Half-life (h)	Treatment	Conc.	Half-life (h)
pH original	L	266.80 ± 6.29	pH 2	L	20.18 ± 0.95
	M	382.86 ± 18.12		M	28.90 ± 1.33
	H	400.75 ± 9.21		H	35.07 ± 0.23
pH 11	L	0.46 ± 0.02	pH 1.5	L	0.13 ± 0.00
	M	0.50 ± 0.02		M	0.13 ± 0.01
	H	0 .57 ± 0.01		H	0.14 ± 0.00
pH 10	L	1.76 ± 0.04	*T* −20	L	4711.01 ± 27.69
	M	1.85 ± 0.05		M	7593.98 ± 100.73
	H	2.05 ± 0.03		H	8111.29 ± 3.02
pH 9	L	54.15 ± 2.43	*T* 4	L	1278.01 ± 51.37
	M	61.12 ± 5.12		M	1689.81 ± 11.42
	H	71.65 ± 3.58		H	1981.55 ± 83.63
pH 8	L	98.33 ± 2.00	*T* 25	L	266.80 ± 6.29
	M	105.35 ± 4.75		M	382.86 ± 18.12
	H	159.77 ± 7.41		H	400.75 ± 9.21
pH 7	L	101.70 ± 5.24	*T* 40	L	1.72 ± 0.11
	M	134.99 ± 4.47		M	2.08 ± 0.12
	H	147.32 ± 9.00		H	2.45 ± 0.17
pH 6	L	357.07 ± 6.42	*T* 55	L	0.73 ± 0.06
	M	374.31 ± 10.76		M	0.90 ± 0.06
	H	402.75 ± 12.86		H	1.08 ± 0.09
pH 5	L	350.28 ± 2.28	*T* 70	L	0.44 ± 0.03
	M	380.22 ± 15.66		M	0.52 ± 0.05
	H	400.22 ± 7.17		H	0.58 ± 0.02
pH 4	L	235.37 ± 8.74	*T* 80	L	0.16 ± 0.00
	M	281.23 ± 11.08		M	0.20 ± 0.03
	H	339.73 ± 3.39		H	0.25 ± 0.01
pH 3	L	39.61 ± 1.86	Light	H	394.15 ± 10.73
	M	45.51 ± 1.43	Dark	H	399.93 ± 8.40
	H	55.42 ± 1.02			

Data are mean ± standard error (SE) of three replicates. H, high concentration (9.03 mg L^{-1}); M, medium concentration (6.02 mg L^{-1}); L, low concentration (3.01 mg L^{-1}).

For 40, 55, 70 and 80 °C, samples were kept in water baths. Allicin residual ratio, degradation fitted curve and half-life calculations were made as above.

Light effects on allicin stability

At 25 °C storage temperature and high allicin concentration (9.03 mg L^{-1}), three replicate samples were kept in an incubator and the stability of allicin in aqueous extract to light was evaluated after 0, 0.5, 1.5, 2, 3, 5, 7, 9, 120, 360, 480 and 720 h. Allicin residual ratio, degradation fitted curve and half-life calculations were made as above.

RESULTS

Quantity of allicin in aqueous extract

Following the UPLC method,[2] the retention time of allicin was around 0.95 min (Fig. 1). The linear equation $y = 874.47x - 408.95$ ($R^2 = 0.9991$) was constructed and the concentration of allicin in samples was calculated from this equation. The concentration of allicin in the original aqueous extract was determined to be 30.11 mg L^{-1}. The pH of the unbuffered aqueous extract was 5.6.

Allicin stability in acid and alkaline solutions

The decrease in allicin stability varied with storage time, concentration and pH (Fig. 2). The stability of allicin in different pH solutions showed a consistent trend at all three concentrations of 9.03, 6.02 and 3.01 mg L^{-1}. Allicin stability in aqueous extract was highest in solutions of pH 5–6, with no obvious degradation for 5 days, but decreased sharply in solutions above pH 11 or below pH 1.5, where complete degradation was observed within 2 h. The stability of allicin in the original aqueous extract was similar with that in solutions of pH 5–6. The half-life of allicin ranged from 235 to 400 h (from 10 to 17 days) at optimal pH 5–6 (Table 1). The rate of degradation of allicin varied slightly with concentration. The data in Table 1 show that the half-lives of allicin at a concentration of 9.03 mg L^{-1} were longer than those at 6.02 mg L^{-1}, which in turn were longer than those at 3.01 mg L^{-1}. This indicated that the half-life of allicin at higher concentration was longer than that at lower concentration.

Allicin stability to storage temperature

As observed in previous studies, allicin was sensitive to storage temperature. At room temperature (25 °C), allicin was stable with no obvious degradation for 5 days (Table 1 and Fig. 3), and its half-life was about 400 h (17 days). However, allicin degraded

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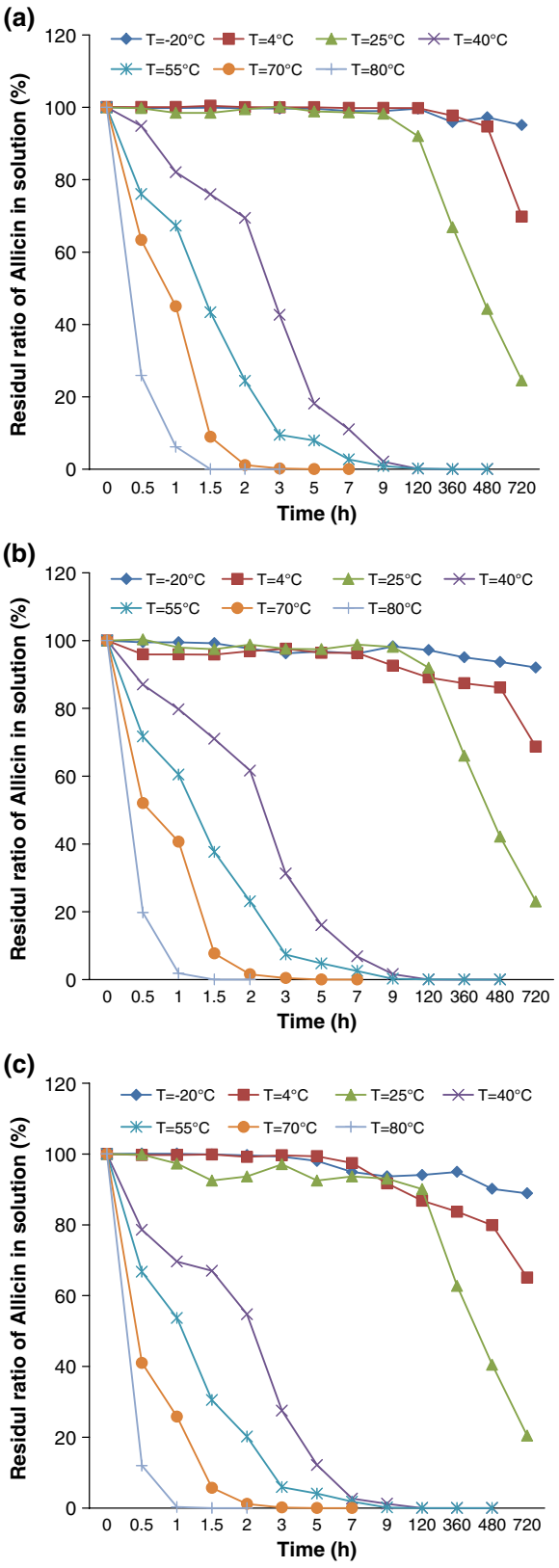

Figure 3. Stability of allicin in aqueous extract as a function of temperature at (a) high (9.03 mg L^{-1}), (b) medium (6.02 mg L^{-1}) and (c) low (3.01 mg L^{-1}) concentrations. Data are mean of three replicates. Error bars denote ± SE.

quickly when the temperature was above 40 °C, with much more rapid degradation above 70 °C. Allicin was stable for at least 20 days at 4 °C and 40 days at −20 °C with no obvious degradation. Allicin degraded completely after 120 h (5 days) when the storage temperature was higher than 40 °C. The longest half-life of about 8100 h (337 days) was observed at −20 °C in the high-concentration solution (9.03 mg L^{-1}). The shortest half-life of only 0.16 h occurred at 80 °C in the lowest concentration solution (3.01 mg L^{-1}).The half-life of allicin at room temperature (25 °C) was around 15 days.

Allicin stability to light

Allicin stability in the original garlic extract remained largely unchanged during storage at 25 °C in either darkness or light (903 µmol m^{-2} s^{-1}). The half-life under light was slightly shorter than that in dark storage (Table 1 and Fig. 4).

DISCUSSION

Allicin, as a principal compound from garlic, has been considered to be a very short-lived substance.[27] According to the results of previous studies, the stability of allicin in aqueous extracts is affected mostly by ambient temperature, pH, solvent and possibly light. In the present study, allicin stability in aqueous extracts was investigated in more detail at different temperatures, pH values, concentrations and light conditions.

This study is the first to evaluate the effects of pH on allicin stability in aqueous extracts. Allicin was very sensitive to pH. Its high sensitivity to low pH confirmed an earlier suggestion that it is difficult for allicin to remain intact in the stomach where the pH is around 2.[31] The stability of allicin in the original aqueous extract was similar to that in solutions with pH 5–6. The half-life of allicin ranged from 10 to 17 days at optimal pH 5–6. It was very unstable in solutions of pH above 11 or below 1.5.

The thermostability measurements obtained over a wide temperature range from −20 to 80 °C in this study suggest that a temperature as low as possible is desirable to preserve allicin in aqueous extracts. The results confirmed previous reports that allicin was unstable at high temperature.[27,32] The half-life of allicin in water was estimated to be 11–16 days at room temperature, which was somewhat longer than the <11.5 days reported by Fujisawa *et al.*[33] but shorter than the 30–40 days reported by Lawson and Gardner.[26] Arzanlou and Bohlooli[6] reported that allicin-free garlic extract was completely degraded to undetectable levels of allicin at room temperature up to 90 days. The inconsistency in half-lives reported by different authors may arise from differences in the concentration of allicin in the extracts or in the room temperature used in the studies.

This study also indicated that the concentration of allicin in solution affects its stability, so that the higher its concentration, the more stable allicin will be. Fujisawa *et al.*[33] suggested that a higher concentration of aqueous extract of garlic would make allicin more unstable. However, in the present study the higher concentration had a longer half-life in both buffer solution and the original aqueous extract. Previous studies have shown a wide genetic variation in the allicin content of garlic, indicating that the allicin contents of different varieties of garlic will be very different.[7,34–37] Thus garlic varieties with high allicin content would be suggested to prepare allicin aqueous extracts.

Allicin is typically stored in low light and is thought by some to be very sensitive to light. However, we found that allicin in aqueous extract was not very sensitive to light, although light exposure did reduce its stability to a small extent. The results reported here were

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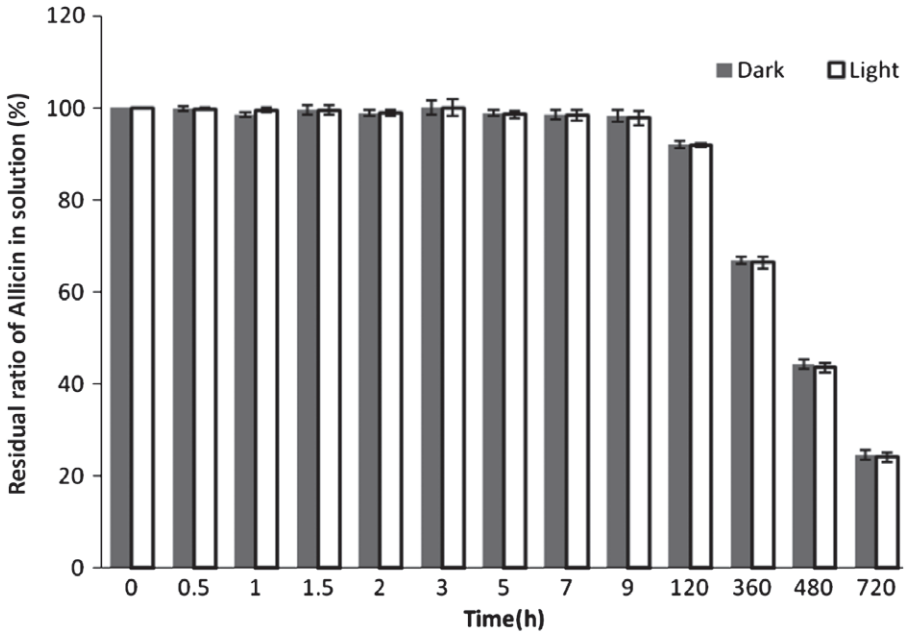

Figure 4. Stability of allicin in aqueous extract under light and dark conditions. Data are mean of three replicates. Error bars denote ± SE.

obtained with light of moderate intensity (903 μmol m^{-2} s^{-1}). Further experiments with stronger light intensity may show greater effects.

CONCLUSIONS

The results obtained in this study indicated that allicin in aqueous extract was sensitive to pH. It was most stable at pH 5–6 but very unstable at pH higher than 11 or lower than 1.5. Allicin in aqueous extract was strongly affected by storage temperature but not to by light. It degraded quickly when the temperature was higher than 40 °C and especially higher than 70 °C. A higher concentration of allicin in solution was more stable. Hence a solution pH of 5–6, a low storage temperature of −20 °C and a dark environment are suggested to retain allicin longer in aqueous extract of garlic.

ACKNOWLEDGEMENTS

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