

## Mechanism of the Greening Color Formation of “Laba” Garlic, a Traditional Homemade Chinese Food Product

BING BAI, FANG CHEN, ZHENGFU WANG, XIAOJUN LIAO,  
 GUANGHUA ZHAO,\* AND XIAOSONG HU

College of Food Science and Nutritional Engineering, China Agricultural University,  
 Beijing 100083, China

While green discoloration during garlic processing is of a major concern, this greening is desirable and required for the traditional homemade Chinese “Laba” garlic. To obtain insights into the mechanism of color formation, simulation of the greening of “Laba” garlic was carried out in the laboratory by soaking aged garlic in 5% (v/v, pH 2.33) acetic acid solution. After 2 days, the garlic cloves turned green. Up to 4 days, pigment(s) diffused from garlic cloves to the pickling solution. The solution exhibits two maximal absorbances at ~440 and ~590 nm, corresponding to yellow and blue species, respectively, the combination of which creates the green coloration. With increasing time from 4 to 25 days, the concentration of both yellow and blue species increases at nearly the same rate, while after 25 days, the concentration of the yellow species increases faster than that of the blue species. Interestingly, most thiosulfinates (~85%) in garlic cloves were converted within 4 days, suggesting that thiosulfinate conversion is proportional to the formation of the pigments. Consistent with this conclusion, alliinase and acetic acid were required for the color formation. UV–vis spectral measurements and pH results suggest that the color formation occurs by two kinds of processes: one enzymatic and the other nonenzymatic. Low pH (2.0–3.0) favors nonenzymatic reactions, while high pH (6.0 or above) is conducive to enzymatic reactions. Thus, the ideal pH for the entire process of garlic greening is between 4.0 and 5.0, which is a compromise of the optimal pH of both the enzymatic and nonenzymatic reactions.

**KEYWORDS:** “Laba” garlic; thiosulfinates; greening; color formation; acetic acid; alliinase

### INTRODUCTION

For more than 4000 years, garlic has been used because of its variety of pharmacological benefits (1–4). Many health properties of garlic are attributed to its organosulfur compounds, particularly thiosulfinates (R-S-S(O)-R'). Diallyl thiosulfinate (allicin) is the most abundant compound, accounting for 60–80% of the overall thiosulfinates present or formed upon crushing garlic cloves (5–8). It is produced by the interaction of the nonprotein amino acid, *S*-allyl-L-cysteine sulfoxide (alliin), with the enzyme, alliinase. One of the most active compounds in garlic, allicin, is known to possess antimicrobial (9), anti-inflammatory (8, 10), antiatherosclerotic (11), and antithrombic (12) properties. It also has the capacity to lower serum lipid levels and blood pressure (5, 13, 14), most of which have been suggested to be related to its strong SH-modifying and anti-oxidant properties (15).

Garlic is processed in various forms such as powder, granules, puree, minced paste, and oleoresin (16–18). During processing and storage of garlic or garlic products, greening is a major concern because it limits commercial utilization and reduces

economic value. However, this discoloration mechanism of garlic is poorly understood. It is proposed that the greening discoloration of garlic purees is similar to the pink discoloration of onion and occurs in two phases (16, 19–21). The first phase corresponds to the production of an ether-soluble organosulfur component (also called “color development”) under the action of alliinase on alk(en)yl-L-cysteine sulfoxides. Subsequently, this component reacts nonenzymatically with amino acid and carbonyl compounds to form pigments. This proposal seems to be supported by recent studies of Kubec et al., showing that, at pH 5.5, a dark blue pigment with a maximal visible absorbance at 582 nm was formed in a system containing *S*-(1-propenyl)-L-cysteine sulfoxide (isoalliin), alliin, glycine, and alliinase (21). Isoalliin is the major nonprotein amino acid occurring in onion and is converted by alliinase to di(1-propenyl) thiosulfinate, which gives onion its flavor. Although isoalliin content is much less than that of alliin in garlic, its key role in the green discoloration of garlic was also observed by Lukes (16).

On the other hand, the development of green color is desirable and required for preparation of “Laba” garlic (“Laba” means 8th of December in the Chinese lunar calendar), a homemade Chinese traditional garlic product, which is made with vinegar during winter. Because it has a green color and unique taste as

\* To whom correspondence should be addressed. Fax: +86-10-62737434. E-mail: gzhao318@yahoo.com.cn.

compared to normal garlic, this kind of garlic is very popular in Northern China and is usually eaten with dumplings for Chinese New Year. It is also known that fresh garlic cannot be used for preparation of "Laba" garlic. Garlic is usually harvested around September in China. An aging process of fresh garlic (corresponding to around a 4-month storage from September to December) is necessary for preparation of "Laba" garlic. Moreover, low temperature is required for triggering this aging process. That is why "Laba" garlic can only be homemade in winter in the north of China. However, similar to the discoloration of garlic during its processing, there is little information on the mechanism of the green color formation of "Laba" garlic.

Whether prevention of the discoloration during garlic processing or understanding the development of the color formation for the preparation of "Laba" garlic, it is important to understand the mechanism of garlic greening. The present study was designed to simulate the aging process of fresh garlic in the laboratory and to determine the mechanism of the green color formation during the "Laba" garlic preparation. Other factors that may cause the greening of garlic were also investigated to further understand the mechanism of this process.

## MATERIALS AND METHODS

**Chemicals.** All solvents/chemicals used were of analytical grade or purer. Hydroxylamine hydrochloride, acetic acid, citric acid, and malic acid were purchased from Beijing Chemistry Co. (Beijing, China). 5,5'-Dithio-bis-(2-nitrobenzoic acid) (DTNB) and L-cysteine were purchased from Sigma Chemical Co. (Beijing, China). The solution of L-cysteine ( $6.0 \times 10^{-4}$  M) and DTNB ( $3.0 \times 10^{-4}$  M) were both freshly prepared in sodium phosphate buffer, and DTNB was stored in a brown reagent bottle at 4 °C after preparation.

**Plant Materials.** Garlic bulbs were obtained from a local market of China Agricultural University. They were harvested in September 2004.

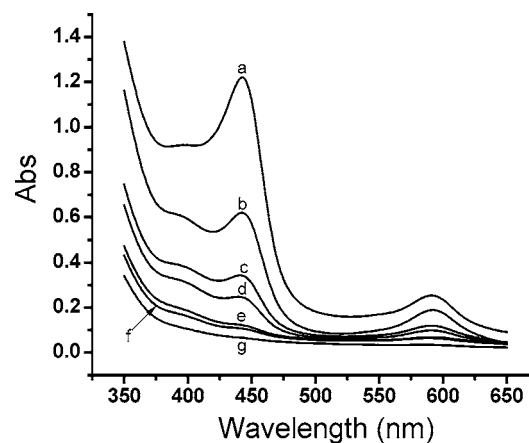
**Preparation of "Laba" Garlic in the Laboratory.** In the laboratory, an aging process was simulated by storing fresh garlic at 4 °C for 1 month. "Laba" garlic production was carried out in the laboratory by soaking the aged garlic in 5% (v/v) acetic acid solution (see below), a result indicating that the simulated aging process is quite similar to the natural aging process.

**Preparation of Garlic-Pickling Solution and Measurement of UV Spectra.** After cracking the aged garlic bulbs, the shriveled, damaged, and small cloves were discarded and the remaining cloves were peeled off, rinsed with tap water, and then triple-rinsed with distilled water 3 times. The cloves were divided into several equal parts (each containing ~30 cloves) and were pickled in acetic acid solutions (5%, v/v) with different pH values from 2.0 to 6.0. The samples were allowed to stand at room temperature for 4–45 days. The resulting green solution was filtered and placed into a quartz cuvette for spectral measurement.

To clarify whether alliinase or thiosulfinate is involved in green color formation of "Laba" garlic, aliquots of garlic cloves were soaked in a series of ethanol solutions [5, 10, 20, 50, and 80 (v/v)], hydroxylamine solutions (0.01, 0.02, 0.1, and 0.5 M), and L-cysteine solutions (0.01, 0.02, 0.05, and 0.1 M), respectively. All of these solution were made with 5% acetic acid solution. After 15 days, their pickling solutions were filtered followed by UV–vis measurements.

**Measurement of the Concentration of Total Thiosulfates in "Laba" Garlic Cloves.** The pickled garlic cloves were taken from the solution, rinsed 3 times with distilled water, and then chopped into 3-mm-thick slices, which were stored in self-sealing bags. After lyophilization for 36 h, the sliced samples were powdered with a mortar and pestle. The powdered samples were kept in a desiccator for future use.

Freeze-dried and powdered garlic samples (~0.1 g) were stirred into 100 mL of 50 mM sodium phosphate buffer (pH 6.9), containing 1 mM EDTA. After 10 min, the solutions were filtrated and total thiosulfates were quantified spectrophotometrically with a UV spectrophotometer TU-1901 (Beijing Spectroscopic Analysis Co.)



**Figure 1.** Family of UV–vis spectra for pickling solution with different times. Condition: 5% acetic acid at pH 2.33. (a) 4 days, (b) 5 days, (c) 7 days, (d) 11 days, (e) 14 days, (f) 25 days, and (g) 45 days.

according to a previous method with slight modification (7). Briefly, a fresh excessive L-cysteine solution was added to the crude garlic extracts. The resulting solution was allowed to stand for 10 min at room temperature followed by addition of the DTNB solution. All solutions of DTNB, L-cysteine, crude garlic extract, and buffer were mixed in equal volumes. This solution produces an absorbance at 412 nm as  $A_1$  with a mixture of DTNB/crude garlic extract/buffer (1:1:2, v/v/v) as a blank. In parallel, a mixture of DTNB/cysteine/buffer (1:1:2, v/v/v) was used as a control and gave an absorbance at 412 nm as  $A_2$  with a mixture of DTNB/buffer (1:3, v/v) as a blank. The content of thiosulfates in garlic was calculated by the formula  $0.5(A_2 - A_1)/f\epsilon$ , where  $f$  is the dilution factor and  $\epsilon = 14\,150\text{ M}^{-1}\text{ cm}^{-1}$  (7).

**Statistical Analysis.** Statistics on a completely randomized design were determined using SAS 9.0 for Windows. Duncan's multiple-range test ( $p < 0.05$ ) was used to determine the significance of differences between means.

## RESULTS AND DISCUSSION

**Kinetic Absorption Spectra for Color Formation.** Similar to green discoloration of garlic purees (16, 22), an aging process at low temperature was also required for preparation of "Laba" garlic. After the aged garlic cloves are soaked in 5% (v/v) acetic acid with nearly identical pH 2.3 as vinegar for 2 days, they become green. Up to 4 days, the pickling solution begins to turn green, and the green color of the solution intensifies up to the 25th day, a result clearly showing that pigment(s) can diffuse from garlic bulb into bulk solution because of its solubility in water. After the garlic solution was filtered in a different time, a family of UV–vis spectra was obtained (Figure 1). There are two maximal absorbances at ~440 and ~590 nm in the visible region, a result suggesting that at least two species exist in the pickling solution; one species has a yellow color, while the other exhibits a blue color. Their combination from both species results in the observed green color of the pickling solution. However, after 25 days, absorbance of the yellow color increases much faster than that of the blue color. From the 25th day to the 45th day, absorbance at 592 nm increases by 33% (from 0.193 to 0.258), whereas the absorbance at 442 nm enhances by 97% (from 0.622 to 1.22). Therefore, after 45 days, the color of the pickling solution is close to yellow rather than green.

**Effect of Acetic Acid on Greening of "Laba" Garlic.** To shed light on the role of acetic acid in the green color formation of "Laba" garlic, two other organic acids were used to pickle the same aged garlic. After incubation for 15 days, the absorbances of their pickling solutions at 590 nm were measured

**Table 1.** Absorbance of Pickling Solution of "Laba" Garlic after a Period of 15 Days at Room Temperature

solution	additives	concentration	abs (590 nm)
acetic acid (5%, v/v)	none		0.2538 ± 0.004 a <sup>a</sup>
	NH <sub>2</sub> OH	0.01 M	0.1909 ± 0.003 d
		0.05 M	0.0415 ± 0.008 f
		0.1 M	0.0134 ± 0.004 h, g
		0.5 M	0.0127 ± 0.002 h, g
	L-cysteine	0.01 M	0.2277 ± 0.009 b
		0.02 M	0.2032 ± 0.002 c
		0.05 M	0.0917 ± 0.006 e
		0.1 M	0.0150 ± 0.003 h, g
	ethanol	5% (v/v)	0.0061 ± 0.0006 h
10% (v/v)		0.0059 ± 0.0004 h	
20% (v/v)		0.0054 ± 0.0009 h	
50% (v/v)		0.0049 ± 0.0008 h	
80% (v/v)		0.0060 ± 0.0011 h	
citric acid		5% (v/v)	0.0145 ± 0.001 h, g
malic acid		5% (v/v)	0.0186 ± 0.001 g

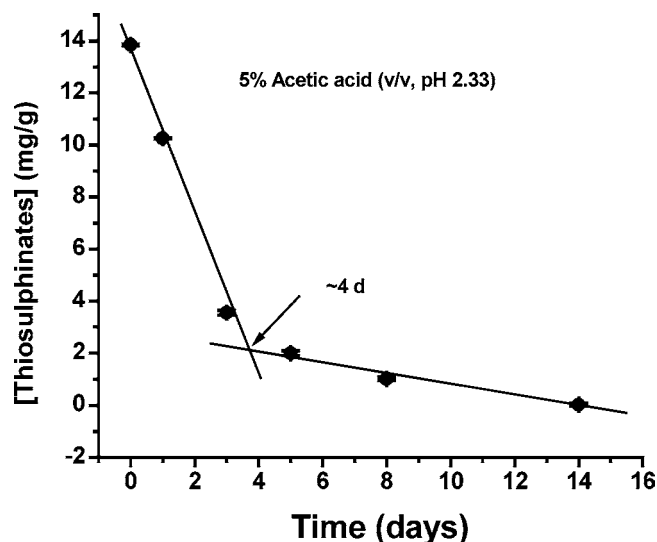
<sup>a</sup> All values are mean ± SD ( $n = 3$ ). Values followed by different letters in the same column are significantly different ( $p < 0.05$ ).

and listed in **Table 1**. The pickling solution containing 5% acetic acid shows the absorbance of 0.2538 at 590 nm, whereas the pickled solution containing 5% citric acid and malic acid exhibits absorbances of 0.0145 and 0.0186 at the same wavelength, respectively. Significant differences in absorbance exhibit between acetic acid solution and two other organic acid solutions ( $p < 0.05$ ). The 590 nm absorbance of the acetic acid solution is ~14–17 times higher than those of the two other solutions. Thus, under the identical conditions, the cloves only in 5% acetic acid become green, while those pickled in both citric acid and malic acid do not turn green. Similarly, the pigment(s) were not formed either when phosphoric acid and chloride acid were substituted for acetic acid to make "Laba" garlic (data not shown). These results suggest that acetic acid is necessary for the green color formation of "Laba" garlic. A more detailed study on the possible mechanism is currently in progress (23).

#### Effect of Alliinase on the Greening of "Laba" Garlic.

Green discoloration of garlic purees was showed to be an alliinase-related process (16, 19–21). This raises a question of whether this enzyme also plays a role in the color formation of "Laba" garlic. To gain insights into the role of alliinase, ethanol (an alliinase denaturant) and NH<sub>2</sub>OH (an inhibitor of alliinase) were added to the pickling solution to study their effects on the green color formation. The results are shown in **Table 1**. As the concentration of inhibitor was increased from 0 to 0.01 to 0.1 M, the absorbance at 590 nm decreased from 0.2538 to 0.1909 to 0.0134, respectively. Complete inhibition was achieved at 0.5 M as suggested by the lowest absorbance, only 0.0127, a result indicating that alliinase is also involved in the color formation of "Laba" garlic. Further supports come from results with ethanol. The absorbance at 590 nm is only 0.0061 in the presence of 5% (v/v) ethanol (**Table 1**), and similar results are obtained when a series of higher ethanol concentrations are used (**Table 1**), suggesting that ethanol can inhibit the color formation, further supporting the above conclusion. Thus, alliinase also participates in the green color formation of "Laba" garlic just as it is required for the greening discoloration during garlic processing (16, 19, 21, 22).

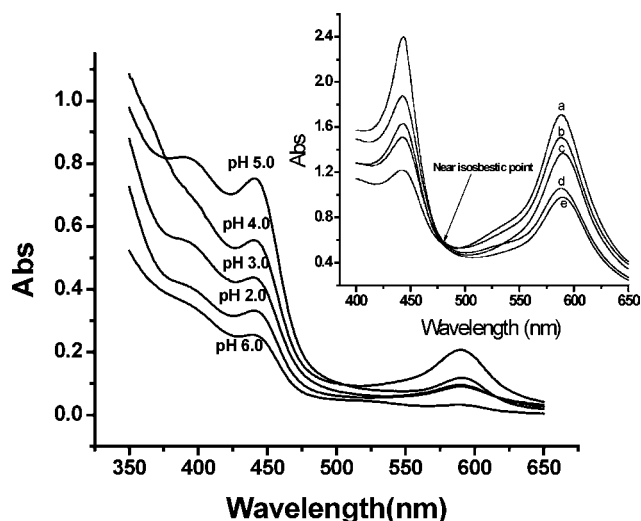
**Participation of Thiosulfinates in the Color Formation of Garlic.** Alliinase is a well-characterized enzyme that catalyzes the conversion of alk(en)yl-L-cysteine sulfoxide(s) to thiosulfinate(s) (4, 24). Previous studies showed that *S*-(1-propenyl)-cysteine sulfoxide (isoalliin) served as the primary precursor for garlic greening (16, 21). Therefore, it is of special interest

**Figure 2.** Plot of total thiosulfinates as a function of time.

whether the products of the action of alliinase on cysteine sulfoxide(s), thiosulfinates, also take part in the discoloration reactions of garlic. To test this idea, the content of total thiosulfinates of garlic bulbs pickled in 5% (v/v) acetic acid (pH 2.33) with different incubation periods (0, 1, 4, 5, 7, and 15 days) was measured (**Figure 2**). Total thiosulfinates in garlic decrease as the incubation time increases. Two phases in the process of the color formation are observed. The first fast phase corresponds to ~85% decrease of total thiosulfinates within 4 days; the second slow phase represents ~15% decrease of total thiosulfinates from 4 to 14 days. In comparison to the production of pigments in garlic (**Figure 1**), it is observed that the decrease in thiosulfinates of garlic is closely associated with the increase of absorbances at ~440 and ~590 nm in the pickling solutions. This result provides direct evidence that the thiosulfinates are involved in the color formation of "Laba" garlic, indicating that the thiosulfinates are the precursors to the pigments. Consistent with this conclusion, the absorbances at ~440 and ~590 nm become observable when most thiosulfinates (85%) were consumed at the 4th day (**Figures 1 and 2**). From the 4th day on, the synthesis of pigments begins at the expense of thiosulfinates, which may correspond to nonenzymatic reactions (see below). In support of this interpretation is the observation showing that, after 14 days, almost all thiosulfinates were used up, but the absorbance at both wavelengths continues to increase (**Figures 1 and 2**).

On the basis of the above results, it is reasonably believed that alliin, the major thiosulfinate in garlic, is involved in the color formation of "Laba" garlic, being in accordance with the recent observation showing that isoalliin and alliin are involved in the blue color formation *in vitro* in the presence of alliinase (21). Because almost all of the thiosulfinates (98.4%) were consumed after 14 days, other thiosulfinates were also involved in the production of the pigments in garlic. This conclusion agreed with the UV-vis measurements showing that at least two pigments developed in the pickling solution (**Figure 1**). Further support for this conclusion comes from the recent report that light blue ( $\lambda_{\max} = 580$  nm) and dark blue ( $\lambda_{\max} = 582$  nm) compounds were prepared *in vitro* by the reaction of thiosulfinates (R-S(O)-S-R', R = allyl, R' = (*E*)- or (*Z*)-propenyl; or R = (*E*)-propenyl, R' = allyl) with glycines at pH 5.0. The blue species with a maximal absorbance at ~590 nm in the present study may be the reaction product of either thiosulfinates



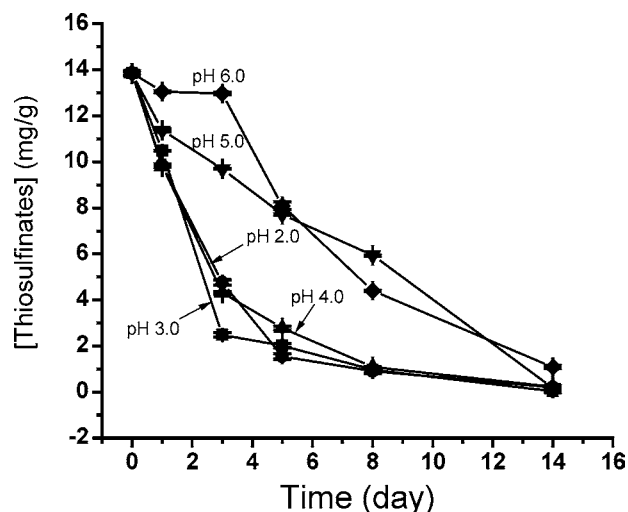


**Figure 3.** Family of UV-vis spectra for pickling solution in the pH range of 2.0–6.0 after 14 days of incubation. (Inset) UV-vis spectra for pickling solution with different time after 7 days of incubation of “Laba” garlic at pH 4. (a) 2 days, (b) 3 days, (c) 5 days, (d) 7 days, and (e) 8 days.

with amino acids rather than glycine or different thiosulfonates with glycine.

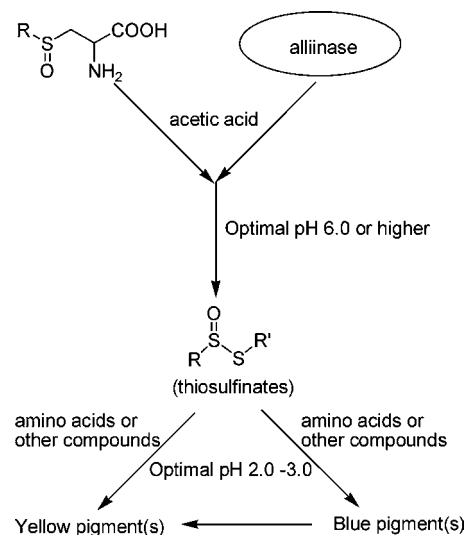
Previous studies showed that greening and browning of various garlic products were suppressed by the addition of L-cysteine (18, 22). The inhibitory effect of cysteine on the color formation was also confirmed by the present study. Garlic color formation was almost completely inhibited by 0.1 M L-cysteine (Table 1). It is known that two molecules of cysteine can react with one molecule of thiosulfonate (7, 8, 15). Because all thiosulfonates were consumed by 0.1 M cysteine, no observable species were produced, again supporting the above conclusion that the thiosulfonates are involved in the formation of the pigments.

**Change of Color Intensities and Total Thiosulfonates as Influenced by pH.** pH has a significant effect on the pink discoloration of onion (25). To determine whether pH also has an effect on the color formation of garlic, the aged garlic was pickled in 5% acetic acid with pH over a range of 2.0–6.0 (Figure 3). All spectra represent separate solution samples and are collected after 14 days. As pH increases from 2.0 to 4.0, the absorbances at both 440 and 590 nm also increase, suggesting that the content of yellow and blue pigments increased with increasing pH over the low pH range. However, as pH increased from 4.0 to 5.0, the 440 nm absorbance increases, whereas the 590 nm absorbance declines, suggesting that pH in the range of 4.0–5.0 facilitates the production of yellow species but inhibits the formation of blue species. Above pH 5.0, the two absorbances decrease, indicating that high pH inhibited the production of both yellow and blue species. A similar pH profile was observed with 25 day samples (data not shown). Because the greatest color formation occurred between 4.0 and 5.0 and the optimal pH for alliinase activity is over a range of 7.0–7.5 (24), the present pH result suggests that the color formation of garlic occurs by two kinds of process; one is enzymatic, and the other is nonenzymatic. Low pH (2.0–3.0) is favored for nonenzymatic reactions, while high pH (6.0 or above) is conducive to enzymatic reactions. Thus, the ideal pH for the entire process of garlic greening is a compromise of the favored pH of both the enzymatic and nonenzymatic reactions. Consistent with this idea, blue compounds were produced *in vitro* at pH 5.0 by reacting thiosulfonates with glycine (21); however, the greatest discoloration of onion took



**Figure 4.** Plot of total thiosulfonates as a function of time as influenced by pH from 2.0 to 6.0.

**Scheme 1.** Proposed Mechanism for the Greening Color Formation of “Laba” Garlic



place at pH ~6 (25), reflecting a slight difference in the color formation mechanism between onion and garlic.

A plot of the content of the thiosulfonates as a function of time with different pH from 2.0 to 6.0 is given in Figure 4. It is observed that pH has a different effect on the decrease of the concentration of the thiosulfonates in garlic. The concentration of the thiosulfonates decreases faster at low pH (2.0–4.0) than at high pH (5.0–6.0), a result suggesting that nonenzymatic reactions favored by low pH are a rate-limiting step.

To illustrate the relationship between yellow and blue species, pickling solution was separated from garlic cloves and its UV-vis spectra were recorded (inset of Figure 3) after garlic cloves were soaked in 5% (v/v) acetic acid at pH 4.0 for 7 days. All spectra represent one sample, which is allowed to stand for different times. It is evident that with increasing time, the ~590 nm absorbance declines, while the ~440 nm absorbance increases, resulting in a near isosbestic point (inset of Figure 3), suggesting that the blue species is replaced by more than yellow species. This result strongly suggests that the yellow species (more than one) are more stable than the blue species and that both kinds of species have similar structures.

## CONCLUSIONS

Our present results indicate that reactions leading to the greening color formation of "Laba" garlic contain at least two steps (**Scheme 1**): (1) the conversion of alk(en)yl-L-cysteine sulfoxide(s) to thiosulfinates catalyzed by alliinase in the presence of acetic acid, the optimal pH of which is 6.0 or higher, and (2) the nonenzymatic synthesis of yellow and blue pigments derived from reactions of the produced thiosulfinates with amino acids or other compounds, which is a rate-limiting step favored by low pH (2.0–3.0).

## ACKNOWLEDGMENT

This project was supported by the Program For New Century Excellent Talents in University (NCET-04-0134). The authors wish to thank Ms. Chris Janus-Chandler for her helpful discussion, suggestions, and corrections.

## LITERATURE CITED

- (1) Das, I.; Hirani, J.; Sooranna, S. Arginine is not responsible for the activation of nitric oxide synthase by garlic. *J. Ethnopharmacol.* **1996**, *53*, 5–9.
- (2) Jia, Z.; Tang, M.; Wu, J. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem.* **1999**, *64*, 555–559.
- (3) de Castro, A.; Montano, A.; Sanchez, A. H.; Rejano, L. Lactic acid fermentation and storage of blanched garlic. *Int. J. Food Microbiol.* **1998**, *39*, 205–211.
- (4) Shen, C.; Parkin, K. L. *In vitro* biogenesis of pure thiosulfinates and propanethial-S-oxide. *J. Agric. Food Chem.* **2000**, *48*, 6254–6260.
- (5) Block, E. The organosulfur chemistry of the genus *Allium*: Implications for the organic chemistry of sulfur. *Angew. Chem. Int. Ed. Engl.* **1992**, *31*, 1135–1178.
- (6) Lawson, L. D. In *Phytochemicals of Europe: Their Chemistry and Biological Activity* (Lawson, L. D., Bauer, R., Eds.) Vol. 691, pp 176–209, American Chemical Society: Washington, DC, 1998.
- (7) Han, J.; Lawson, L.; Han, G.; Han, P. A spectrophotometric method for quantitative determination of allicin and total garlic thiosulfinates. *Anal. Biochem.* **1995**, *225*, 157–160.
- (8) Miron, T.; Rabinkov, A.; Mirelman, D.; Weiner, L.; Wilchek, M. A spectrophotometric assay for allicin and alliinase (alliin lyase) activity: Reaction of 2-nitro-5-thiobenzonate with thiosulfinates. *Anal. Biochem.* **1998**, *265*, 317–325.
- (9) Ankri, S.; Mirelman, D. Antimicrobial properties of allicin from garlic. *Microbes Infect.* **1999**, *2*, 125–129.
- (10) Mayeux, P. R.; Agrawal, K. C.; Tou, J. S.; King, B. T.; Lippton, H. L.; Hyman, A. L.; Kadowitz, P. J.; McNamara, D. B. The pharmacological effects of allicin, a constituent of garlic oil. *Agents Actions* **1998**, *25*, 182–190.
- (11) Abramovitz, D.; Gavri, S.; Harats, D.; Levkovitz, H.; Mirelman, D.; Miron, T.; Eilat-Adar, S.; Rabinkov, A.; Wilchek, M.; Eldar, M.; Vered, Z. Allicin-induced decrease in formation of fatty streaks (atherosclerosis) in mice fed a cholesterol-rich diet. *Coron. Artery Dis.* **1999**, *10*, 515–519.
- (12) Lawson, L. D.; Ransom, D. K.; Hughes, B. G. Inhibition of whole blood platelet-aggregation by compounds in garlic clove extracts and commercial garlic products. *Thromb. Res.* **1992**, *65*, 141–156.
- (13) Augusti, K. T.; Mathew, P. T. Lipid lowering effect of allicin (diallyl disulfide oxide) on long-term feeding to normal rats. *Experientia* **1974**, *30*, 468–470.
- (14) Agarwal, K. C. Therapeutic actions of garlic constituents. *Med. Res. Rev.* **1996**, *16*, 111–124.
- (15) Rabinkov, A.; Miron, T.; Mirelman, D.; Wilchek, M.; Glozman, S.; Yavin, E.; Weiner, L. S-Allylmercaptogluthathione: The reaction product of allicin with glutathione possesses SH-modifying and antioxidant properties. *Biochim. Biophys. Acta* **2000**, *1499*, 144–153.
- (16) Lukes, T. M. Factors governing the greening of garlic puree. *J. Food Sci.* **1986**, *51*, 1581–1582.
- (17) Joslyn, M. A.; Sano, T. The formation and decomposition of green pigment in crushed garlic tissue. *Food Res.* **1956**, *21*, 170–183.
- (18) Kim, W. J.; Cho, J. S.; Kim, K. H. Stabilization of ground garlic color by cysteine, ascorbic acid, trisodium phosphate, and sodium metabisulfite. *J. Food Qual.* **1999**, *22*, 681–691.
- (19) Shannon, S.; Yamaguchi, M.; Howard, F. D. Precursors involved in the formation of pink pigments in onion purees. *J. Agric. Food Chem.* **1967**, *15*, 423–426.
- (20) Shannon, S.; Yamaguchi, M.; Howard, F. D. Reactions involved in formation of a pink pigment in onion purees. *J. Agric. Food Chem.* **1967**, *15*, 417–422.
- (21) Kubec, R.; Hrbacova, M.; Musah, R. A.; Velisek, J. *Allium* discoloration: Precursors involved in onion pinking and garlic greening. *J. Agric. Food Chem.* **2004**, *52*, 5089–5094.
- (22) Jiang, Y.; Hu, X.; Xin, L.; Liu, Y. Control of greening in garlic puree. *Food Sci.* **2001**, *22*, 55–56.
- (23) Li, L. M.S. Thesis, Study of mechanism of the color formation of "Laba" garlic, a Chinese traditional food product. China Agricultural University, Beijing, China, 2005.
- (24) Krest, I.; Glodek, J.; Keusgen, M. Cysteine sulfoxides and alliinase activity of some *Allium* species. *J. Agric. Food Chem.* **2000**, *48*, 3753–3760.
- (25) Lee, C.-H.; Parkin, K. L. Relationship between thiosulfinates and pink discoloration in onion extracts, as influenced by pH. *Food Chem.* **1998**, *61*, 345–350.

---

Received for review May 25, 2005. Revised manuscript received July 12, 2005. Accepted July 14, 2005. This project was supported by the Program for New Century Excellent Talents in University (NCET-04-0134).

JF051211W