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Correlation between Pungency and Allicin Content of Pickled Garlic during Aging

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Abstract

Relationship between pungency and allicin content of pickled garlic during aging was examined. Degree of pungency of pickled garlic during aging at 20° C was determined by the sensory evaluation. A panel of 10 members evaluated seven samples of pickled garlic which were aged for 0, 10, 20, 30, 40, 50 or 60 days by using scoring test (seven point scale). The sensory evaluation results showed that pungency of pickled garlic decreased gradually during aging, and scored at 3.07 on the 40th day of aging. Content of allicin, which was a major pungent component of garlic homogenate, was quantitatively analyzed by HPLC. The level of allicin in homogenate of pickled garlic was found to decrease gradually, and to 5.9% on the 40th day of aging compared with that of fresh garlic. Relationship between the pungency score results and the content of allicin demonstrated a highly positive correlation (r=0.9648).

Key words : pickled garlic, pungency, sensory evaluation, allicin

INTRODUCTION

The characteristic pungent flavor of garlic (*Alliium sativum* L.) has been ascribed to sulfur containing compounds. In 1944, Cavallito and Bailey isolated colorless oil from garlic[1]. They investigated the chemical and antibacterial properties of this new substance, which they named allicin. On the basis of chemical observations, Cavallito *et al.* proposed a diallythiosulfinate structure for allicin[2]. Stoll and Seebeck demonstrated that allicin is produced from alliin as a major flavor precursor following injury through the action of the enzyme alliinase[3]. Also, they reported that intact garlic cloves contain 0.24% by weight S-allyl-L-cysteine sulfoxide (alliin), a colorless and odorless solid[3]. Fujiwara *et al.*[4] reported the presence of methyl, methylpropyl, propyl, methylallyl, propylallyl and allyl allicin in garlic. Later investigations confirmed S-allylcysteine sulfoxide as a major flavor precursor in garlic, with trace amounts of methyl and propyl derivatives[5]. These sulfoxides are enzymatically converted into the thiosulfinates, which non-enzymatically disproportionate to from symmetrical and mixed mono-, di- and trisulfides, as well as sulfur dioxide[6,7]. Saghir *et al.*[8] assigned the odor of fresh garlic to allicin and the cooked odor was attributed to di- and trisulfides. Brodnitz *et al.*[7] also found allicin to be a major volatile component in freshly chopped garlic and to rearrange on the GC column giving rise to 3-vinyl-1,2-dithi-4-ene and 3-vinyl-1,2-dithi-5-ene. For the analysis of allicin or thiosulfinates, determinations by paper chromatography[4] and colorimetry[6,9-11] had been used. However, the disadvantages of these color reactions are the instability of the colored compounds and, in addition, the fact that pyruvic acid is formed by enzymatic cleavage of all the different alkylcysteine sulfoxides present in garlic. Due to the thermal instability of allicin, gas chromatographic and GC-MS analysis result in disintegration products such as di- and trisulfides[7,8]. Recently, Jansen[12] developed a HPLC assay to quantitatively determine allicin, which represents one main marker for the evaluation of garlic.

Pickled garlic, which has been cherished as a favorite food, is known to be devoid of a strong pungent odor of fresh garlic. However, there is no report on

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the change in pungency of pickled garlic. This research was undertaken to demonstrate the relationship between the pungency score of sensory test and the amount of allicin in homogenates of pickled garlic during aging.

MATERIALS AND METHODS

Materials

Fresh garlic was purchased at a local market in Taejon. S-Ethyl-L-cysteine and diallyldisulfide were obtained from Fluka, conconavalin A-Sepharose (con A-Sepharose), hydroxylapatite, phenylmethyl sulfonyl fluoride (PMSF), 5' -pyridoxal phosphate, polyvinyl polypyrolidone (PVPP), bovine serum albumine, α-methyl-D-manopyranoside, cellulose (type 20) and ninhydrin were obtained from Sigma Chem Co. All other reagents were of anaiytical grade.

Preparation of pickled garlic

Peeled fresh garlic cloves (50g) were submerged in 100ml of 2% acetic acid containing 2% NaCl, and were aged at 20° C for 60 days in a closed state.

Samples were analyzed in triplicate at the start and after 10, 20, 30, 40, 50 and 60 days of aging.

pH and acidity of pickled garlic

pH and titrable acidity were measured periodically during aging according to the AOAC[13]. Solutions were directly assayed, and tissues (50g) were analyzed after the homogenzation in distilled water (100ml) using Waring blendor and the filtration through filter paper (Toyo No. 5).

Determination of allicin content

HPLC assay for allicin : HPLC assay for allicin was carried out according to a modified Jansen's method [12]. Fresh or pickled garlic cloves (5g) were homogenized in distilled water to give a final volumn of 30ml. The suspension was incubated in a closed tube for 20min at 30° C and then centrifuged at 15,000 × g for 20min. Methanol (1.5ml) were added to 1ml of the supernatant. The resulting suspension was centrifuged at 8,000 × g for 5min. The supernatant (2.5ml) was mixed with benzene (5μl) as an internal standard. The mixture (15μl) was injected onto a C_{18} μ Bondapak column (10μm, 39 × 300mm, Waters Assoc.). The eluant was methanol (60%) in water containing 0.1% formic acid, and the flow rate was 0.8ml/min. Detection was carried out with a model M486 UV detector (Waters Assoc.) at 254nm. The amount of allicin was determined from the calibration curve, which was constructed by plotting the peak area ratios of allicin to benzene versus the concentration of allicin.

Preparation of alliin from garlic : Isolation of alliin was carried out according to Stoll and Seebeck[11]. The garlic cloves were deep-frozen for 2 days at −70° C, and crumbled to a fine powder while frozen in a Waring blendor with 2kg of dry ice per 500g cloves. Powdered sample was extracted with 1.5 liter of absolute methanol for 1hour in a shaking bath, and filtered under vacuum with Toyo No. 5 filter paper. The residue was reextracted twice with 1 liter of 80% (v/v) methanol, and the combined extract solutions was evaporated under reduced pressure until yellowish and syrupy liquid was formed. Syrupy liquid was again extracted with ether to eliminate the fatty component and impurities, and dried under vacuum pressure. The yellowish powder was dissolved in distilled water (80ml), and mixed with 300ml of absolute ethanol. The turbid solution was stood for 10 hours at 4° C, and then the supernatant was cautiously decanted from the precipitate containing carbohydrates. The same procedures were repeated two or three times with additional ethanol. Final solution was concentrated under reduced pressure, and cold methanol was added to the concentrated aqueous solution. The resulting white precipitates were dissolved in 6ml of distilled water, and then 12ml of hot acetone (50° C) was added to the above solution. During cooling, needlish-bundled crystals were fromed.

Preparation of purified alliinase : Preparation of purified alliinase was carried out using affinity chromatograpy according to Nock and Mazelis[14]. Peeled (250g) and chopped garlic cloves were blended for 1-min in a cold buffer of 20mM phosphate, pH 7.0 containing 10% glycerol, 1mM PMSF, 5mM EDTA, 5% PVPP and 0.05% 2-mercaptoethanol. The homogenate was filtered, and the filtrate centrifuged (16,300 × g, 1hr) at 40° C. The supernation was brought to

35% saturation with $(NH_4)_2SO_4$. The pellet after centrifugation (16,000×g 30min) was resuspended in approximately 100ml of 0.05M, pH 7, phosphate buffer containing 10% glycerol, 1mM PMSF and 5mM EDTA, and then dialyzed against the same buffer. The dialyzate was centrifuged (12,000×g, 30min), and the supernatant was then applied onto a fast flow hydroxylapatite column (2.2×50cm), which was eluted with 3 volumes of 0.3M buffer. Tubes containing the enzyme activity were combined, concentrated by ultrafiltration, then dialyzed. The concentrate was applied onto a con A-Sepharose 4B column (1×20cm), which was eluted using a gradient of 0 to 100mM methyl-α-D-mannopyranoside in the phosphate buffer. The amount of protein was determined by Lowry's method[15]. The standard enzyme assay mixture (1ml) consisted of 100mM phosphate buffer (pH 6.5), 0.025mM pyridoxal-5'-phosphate and 40mM S-ethyl-L-cysteine sulfoxide. The enzyme reaction was performed at room temperature for 5min, and then terminated by adding 2ml of 10% (w/v) TCA. Aliquots of this final solution were assayed for pyruvate by the total keto acid method of Friedemann and Haugen[16].

Identification of allicin produced from alliin-alliinase system : Alliin, which was isolated from fresh garlic, was dissolved in water, and subjected to TLC as described in Table 1. After developing in solvent system, ninhydrin test was carried out. Each zone corresponds to a ninhydrin-positive spot was scraped off, and dissolved in phosphate buffer. After centrifuged (4,000rpm, 40min), the supernatant was mixed with 25μM of pyridoxal-5'-phosphate and 200 units of purified alliinase. After 5min incubation, the reaction was stopped by adding methanol (1.83ml), and the reaction mixture was analyzed by HPLC. The fraction of each peak was collected and was subjected to pungency test.

Table 1. TLC preparation

Plate	20×15cm glass plate are spread with a slurry of 25% cellulose (Sigmacell type 20, 20μm) in distilled water, dried at room temperature and used without heating
Developing solvent	t-butylalcohol : formic acid 85% : conc. HCL : water (95 : 15 : 18 : 12) by volume)
Color developing solvent	0.5g ninhydrin in a mixture of 10 parts of glacial acetic acid, 20 parts pyridine, 30 parts methylcellosolve, 40 parts t-butylalcohol

Sensory evaluation

Pickled garlic aged for 0, 10, 20, 30, 40, 50 and 60 days at 20° C were evaluated according to Meilgaard *et al.*[17]. Seven samples (50g) were placed in glass bowls and were coded with a three-digit random number and served in a random order to an experienced 10 members who were selected from faculty and graduate students of the College of Home Economics. In our test, judges who dislike strong pungent taste of garlic were excluded by performing two sets of preliminary sensory test. Evaluation of samples were conducted duplicate in individual booths at 10 A. M. and 3 P. M. The following palatability traits were scored using a 7-point scale : Acidity and pungency, 7=very strong, 1=very weak ; over-all acceptability, 7=very good, 1=very poor. The data was statistically analyzed using the analysis of variance. Duncan's multiple range test were used to determine significance among means[18].

RESULTS AND DISCUSSION

The sensory quality of the pickled garlic during aging is shown in Table 2.

It was found that 40 day aging was appropriate for good over-all acceptability, which requires the combination of palatability traits such as fresh sour taste, loss of pungent taste, high fracturability. The pungency of pickled garlic decreased gradually, and scored

Table 2. Mean score values for pungency, acidity and over-all acceptability of pickled garlic during aging at 20° C

Characteristics	Aging time (days)						
	0	10	20	30	40	50	60
Pungency	7.00^{a*}	6.14^{b}	5.07^{c}	4.00^{d}	3.07^{e}	2.00^{f}	1.21^{g}
Acidity	1.14^{f}	2.43^{e}	3.14^{cde}	4.14^{c}	5.36^{ab}	5.50^{a}	3.77^{cd}
Over-all acceptability	1.07^{f}	2.07^{ef}	3.07^{e}	5.00^{abc}	5.93^{a}	5.14^{ab}	4.57^{bcd}

*Any two means in the same rows followed by the same superscripts are not significantly different ($p<0.01$) to Duncan's multiple range test

at 3.07 on 40th day of aging. Therefore, it was assumed that the decrease of pungency contributed to better sensory quality. The degree of pungency in the sensory test was thought to correlate with the amount of allicin in the homogenate of garlic clove, because allicin, which is rapidly formed from the enzymatic hydrolysis of alliin, is known to be a major pungent component in garlic[7,8].

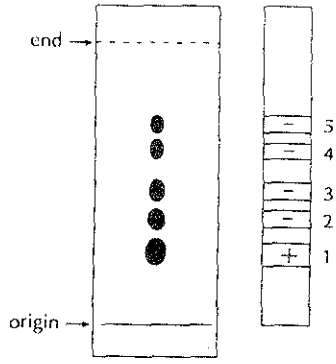

Fig. 1. A. TLC of alliin isolated from fresh garlic. B. Pungency test : Each spot scraped off and treated with enzyme and subjected to sensory test.

In this respect, we attempted to measure the content of allicin in the tissue of garlic clove. The preparation of natural alliin was performed as reported by Stoll and Seebeck[3], and alliin was identified by TLC analysis, which was accompanied by a pungency test after alliinase treatment. Fig. 1 demonstrates the TLC profile of the products isolated from fresh garlic. The spot with a Rf value of 0.2 was found to be close to the Rf value reported for alliin[19]. In the related experiment, where each spot was scraped off and treated with the purified alliinase, a typical pungent smell was observed with the sample from only a spot showing a Rf value of 2.0.

Next, the separation on allicin was performed using HPLC analysis coupled with alliinase treatment. Fig. 2 shows a profile of HPLC chromatogram of the product after treatment of alliin with alliinase. In this chromatogram, peak with a RT of 7.08 min was found to be a product from an enzymatic hydrolysis of alliin. The fresh garlic extract was subjected to HPLC

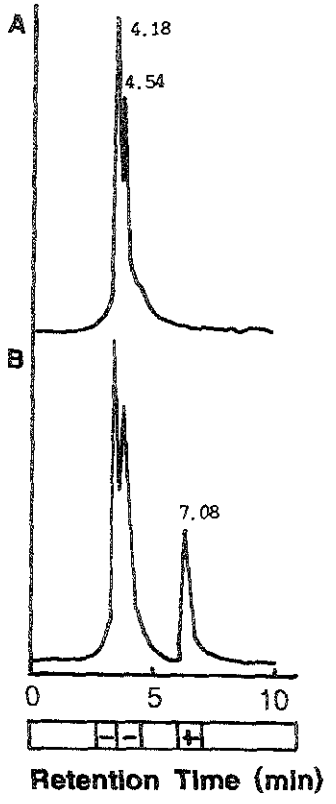

Fig. 2. HPLC chromatogram (A) before (B) after alliinase treatment on isolated alliin(spot 1 of TLC).

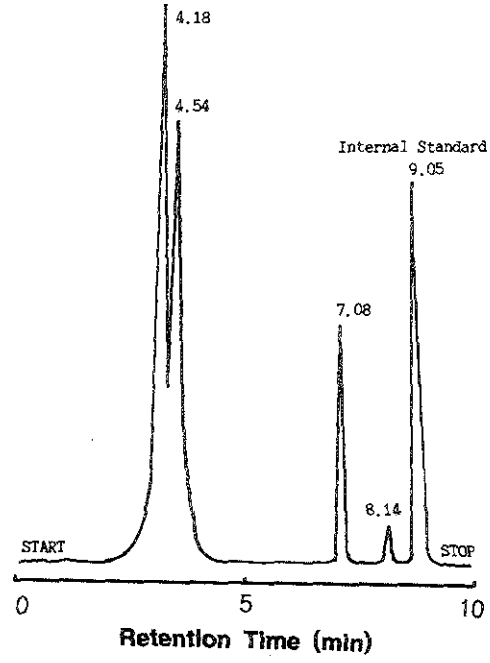

Fig. 3. A. HPLC chromatogram of fresh garlic extract. B. Pungency of fractionated peaks by smelling test.

analysis as described above, a similar HPLC chromatogram was obtained as in Fig. 3. Each fraction was collected and used for smelling test. As might be expected, a fraction with a RT 7.02 min possessed a pungency of fresh garlic, suggesting that it may contain allicin. In addition, UV spectra of the fraction with a RT of 7.02 min was consistent with that reported previously[20]. Based on these results, we established a HPLC method to quantitate allicin relatively. Fig. 4. demonstrates a typical chromatogram of allicin and benzene as an internal standard. The relative amount of allicin was calculated from a calibration curve, which was constructed by plotting the peak area ratio of allicin to benzene versus the peak area of allicin. This calibration curve showed a good linearity (correlation coefficient, γ=0.9991). When allicin in the homogenate of pickled garlic was determined by HPLC, the aging time-dependent decrease in the level of allicin was observed (Fig. 5). On the 40th day of aging, the level of allicin dropped to about 5.9%, compared with that of fresh garlic.

When the relationship between the relative amount of allicin and the pungency score was examined, there was a good correlationship (γ=0.9648) as depicted in Fig. 6. These results indicate that the gradual loss of pungency in pickled garlic is due mainly to the

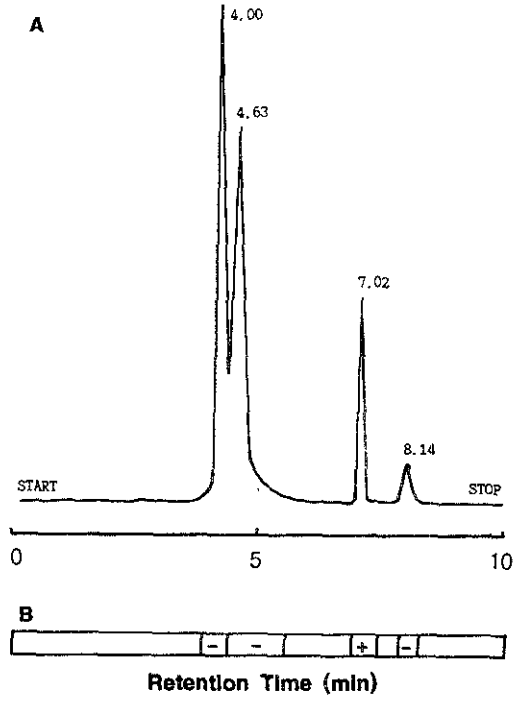

Fig. 4. HPLC chromatogram of benzene as internal standard.

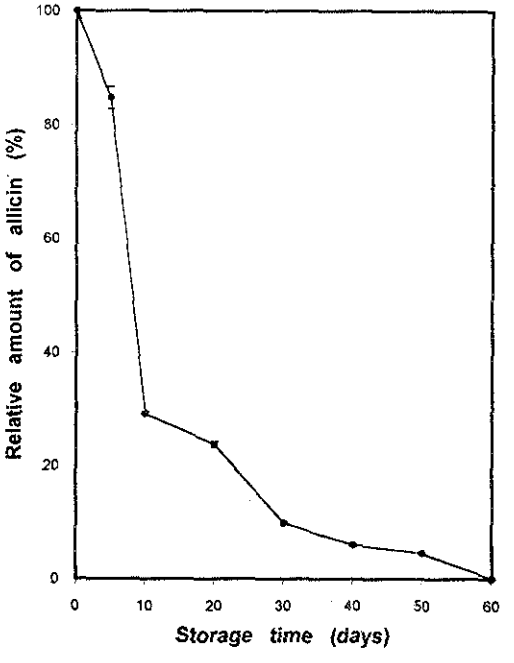

Fig. 5. Change in allicin content of pickled garlic homogenate during aging at 20°C.

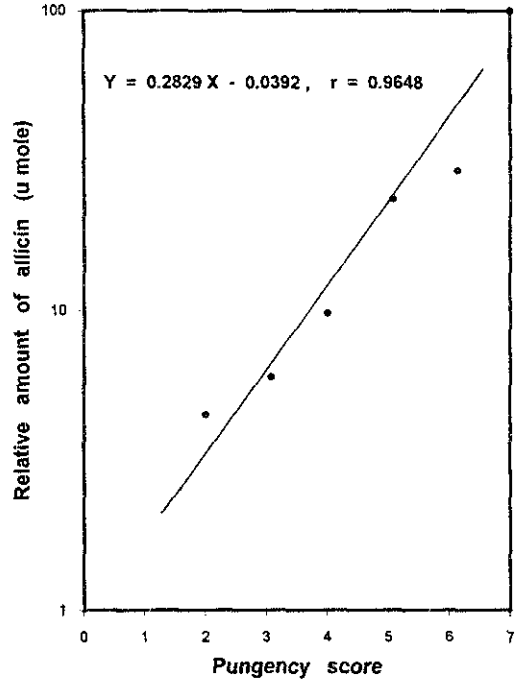

Fig. 6. Relationship between pungency score of sensory evaluation and amount of allicin.

decreased formation of allicin in the tissue of the pickled garlic during aging. Further study remains to be done to see causes for the decrease in pungency of pickled garlic.

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마늘장아찌 숙성 중 매운맛과 Allicin량과의 상관관계

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요 약

마늘장아찌 숙성 중 매운맛과 allicin량과의 상관관계를 살펴보았다. 마늘장아찌의 매운맛은 10명의 관능검사 요원이 7점 만점의 scoring test에 의해 장아찌를 담근 직 후 부터 60일 까지 숙성시키는 동안 10일 간격의 시료에 대해 관능검사를 실시하였다. 관능검사 결과, 숙성 중 마늘장아찌의 매운맛은 서서히 감소하여 40일째에 3.07점이었다. 마늘 homogenate의 주 매운성분인 allicin량은 HPLC에 의해 분석하였으며, 마늘 장아찌가 숙성되는 동안 allicin량이 감소하여, 숙성 40일에는 생마늘에 비해 5.9%로 감소되었다. 관능검사 결과의 매운맛 점수와 allicin량과는 양의 상관관계를 나타내었다 (r=0.9648).