

Article

Identifying the Garlic and Grain Mixture with the Highest Allithiamine Content

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Abstract: Garlic (*Allium sativum* L.) has been extensively studied for its therapeutic and culinary applications, owing to its sulfur-containing bioactive compounds, including allicin and its derivatives. This study identified garlic varieties with high allicin content from different regions of Korea. It explores the synthesis of allithiamine, a lipid-soluble derivative of thiamine with enhanced bioavailability, by combining garlic with various grains. High-performance liquid chromatography (HPLC) analysis revealed significant regional variations in the allicin content, with Jeju garlic exhibiting the highest levels (1.04 mg/g). Among the grains tested, *Avena sativa* showed the most effective interaction with garlic, yielding the highest allithiamine levels (14.93 mg/g). These findings underscore the importance of grain matrix properties in optimizing the synthesis of allithiamine. This study provides valuable insights into the development of functional foods that leverage the bioactive compounds in garlic to enhance metabolic health and thiamine bioavailability.

Keywords: *Allium sativum*; allicin; allithiamine; grain; thiamine



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1. Introduction

Garlic (*Allium sativum* L.) has been valued for its culinary and medicinal properties for decades. The effects of *A. sativum* extracts and their bioactive compounds are being studied for the phytotherapeutic properties of *Allium* species, and several bioactive compounds have shown diverse health benefits [1,2]. *A. sativum* is one of the most widely cultivated therapeutic plants [3]. It contains bioactive compounds such as alliin (S-allyl-cysteine sulfoxide), allicin (diallyl disulfide oxide), diallyl sulfides, S-allyl-cysteine, and ajoene [4]. Levels of alliin in *A. sativum* are high [5]. This precursor undergoes enzymatic conversion during crushing or cutting [6]. In addition to garlic, most grains hold immense nutritional significance as a source of energy and micronutrients, particularly thiamine (vitamin B1). Thiamine plays a crucial role in energy metabolism by functioning as a coenzyme for key enzymatic reactions [7,8]. The enzyme allinase acts on alliin, producing allicin, pyruvate, and ammonia [9]. Allicin is a highly unstable compound that quickly transforms into various sulfur-containing compounds such as diallyl sulfide, dimethyl trisulfide, diallyl disulfide, and diallyl trisulfide, which contribute to its characteristic pungent aroma and flavor [6]. These sulfur compounds, along with allicin, are known for their biological activities [10–12], associated with numerous health-promoting effects, including antioxidative [13], hypoglycemic, and cardioprotective effects [14,15] and anticancer [16], antifibrotic [17], and

antifungal properties [18]. Garlic demonstrated a significant vasodilatory effect on the coronary arteries, leading to increased coronary blood flow in the experimental group before and during reperfusion. This effect was attributed to a reduced concentration of the released LDH [19].

The reaction between allicin and thiamine (Figure 1) produces allithiamine (also known as thiamine-allyl disulfide). Allithiamine, a lipid-soluble form of vitamin B₁, was first discovered in *A. sativum* in the 1950s [20]. This natural derivative of thiamine is unique due to its fat solubility, enhancing its absorption in the intestines and slowing its excretion from the body [21]. This makes allithiamine more bioavailable than water-soluble thiamine, allowing it to be absorbed more effectively in the digestive tract [22]. Allithiamine has been shown to have demonstrated significant potential as an active vitamin for treating conditions like sepsis [23]. The combination of garlic and grains provides a novel approach for creating functional foods with enhanced health benefits by leveraging the sulfur-based bioactive compounds in garlic and the thiamine content in grains. However, the grain matrix properties and their interaction with allicin play a critical role in optimizing allithiamine synthesis, necessitating further research.

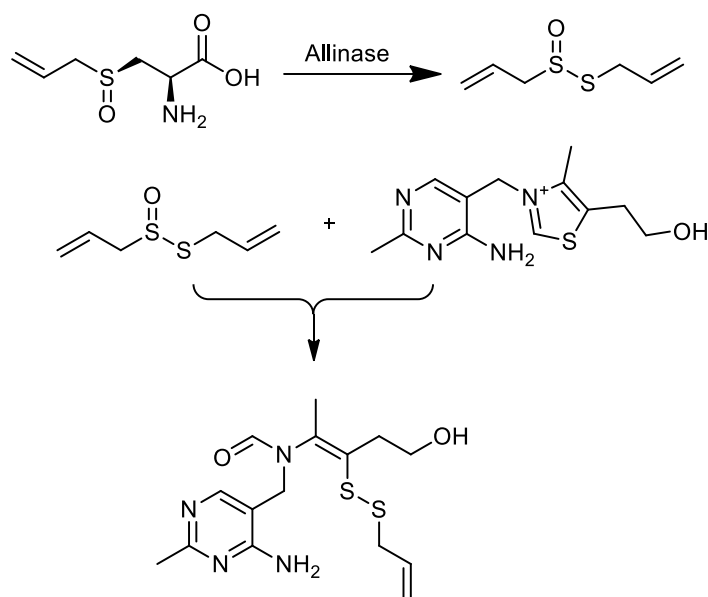


Figure 1. Biosynthetic pathway of allithiamine formation from alliin via allicin and thiamine.

This preliminary study aimed to further enhance the utility of garlic by identifying high-allicin garlic varieties sourced from different regions of Korea. In addition, this study investigated the production of allithiamine by combining garlic with various grain samples to optimize its content, providing a novel approach for maximizing the functional applications of garlic.

2. Materials and Methods

2.1. Plant Materials

Garlic (Figure 2) was collected from different regions of Korea (Jeju garlic from Jeju Agricultural, Uiseong garlic (Hanji-type) from Yeongtae F&B, Yeongcheon garlic (Daeseo-type) from Yeongcheon Yellow Garlic, Namhae garlic (Namdo-type) from Namhae Agricultural Marine Products Direct Sales Center, and Muan garlic (Daeseo-type) from Hwangtotosarang Distribution). All garlic samples were deposited in Nanum Pharmaceutical Co., Ltd. (Yeongcheon, Republic of Korea). The different grain samples *Avena sativa*, *Arachis hypogaea*, *Phaseolus vulgaris*, and *Rhynchosia nulubilis* (Figure 3) were purchased from Taebok Japgok, Kyoungpook Yeongcheon Market, Republic of Korea. A thorough investigation

was conducted to identify native grains with high thiamine content, and these four species, known for their nutritional value, were chosen for their relevance to this study.

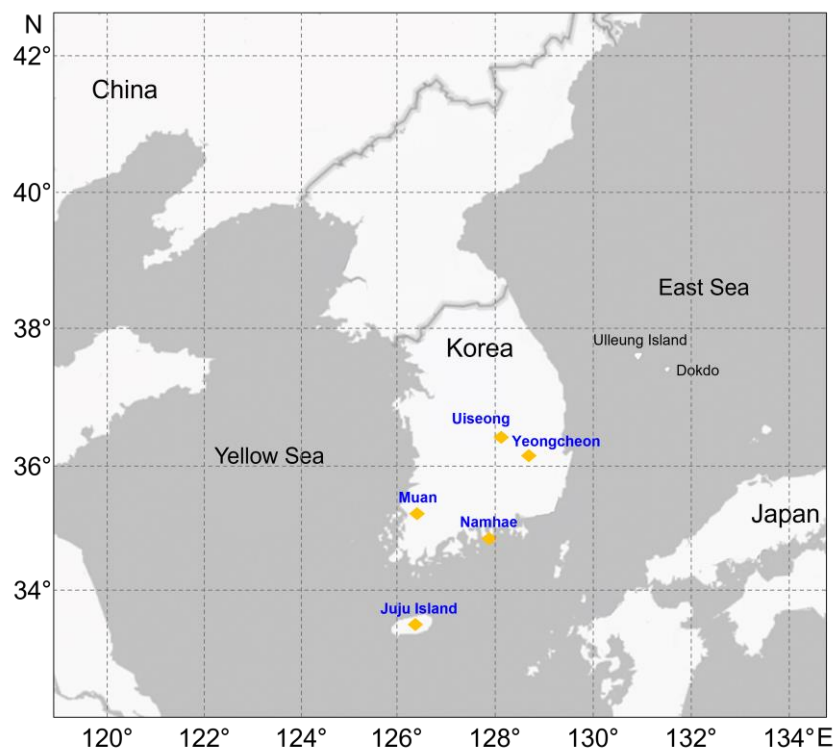


Figure 2. Collection sites of garlic samples.

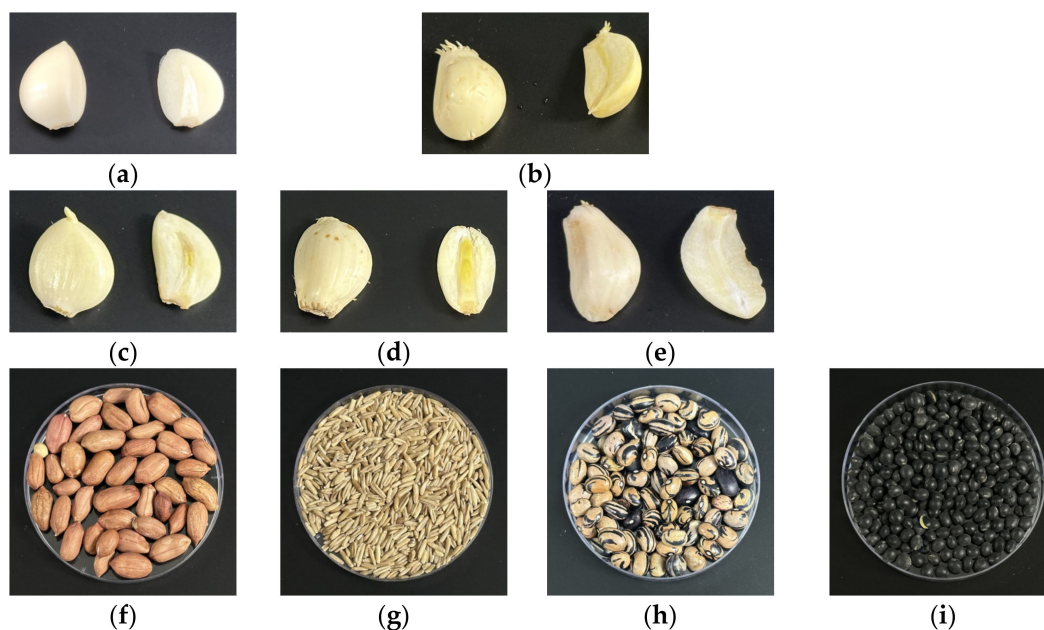


Figure 3. The garlic and grain raw materials, Jeju garlic (a), Muan garlic (b), Namhae garlic (c), Uiseong garlic (d), Yeongcheon garlic (e), *A. hypogaea* (f), *A. sativa* (g), *P. vulgaris* (h), and *R. nulubilis* (i).

2.2. Equipment and Reagents

The grain mixtures were prepared using a grinder (RT-02A, Rong Tsong Precision Technology Co., Ltd., Taichung, Taiwan) and a garlic press (KHC-1000G; Kitchen Art Co., Ltd., Shenzhen, China) to ensure uniform particle size and consistency. An HPLC system (Agilent 1260 Infinity II Quat Pump, Santa Clara, CA, USA) consisting of an autosampler,

a pump, and a Variable Wavelength (VW) Detector (Santa Clara, CA, USA) was used for accurate quantitative analysis. Separation was performed using two columns: a YMC Pack Pro C18 column (4.6×250 mm, $5 \mu\text{m}$) (YMC Co., Ltd., Kyoto, Japan) and an Aegispak C18-L column (3.0×150 mm, $5 \mu\text{m}$) (Young Jin Biochrom Co., Ltd., Seongnam, Republic of Korea). HPLC-grade solvents, including water and methanol (MeOH), were obtained from Honeywell (Burdick and Jackson, Muskegon, MI, USA), while acetonitrile (ACN) was sourced from Scharlau (Barcelona, Spain). Formic and phosphoric acids (Thermo Fisher Scientific, Waltham, MA, USA) were used as the mobile phase additives. The standard compounds, allicin (mixture of diallyl sulfide, DAS; dimethyl trisulfide, DAT; diallyl disulfide, DADS; and diallyl trisulfide, DATS), thiamine, and allithiamine, were procured from the Natural Product Institute of Science and Technology (www.nist.re.kr, accessed on 10 July 2024), Anseong, Republic of Korea (Figure 4).

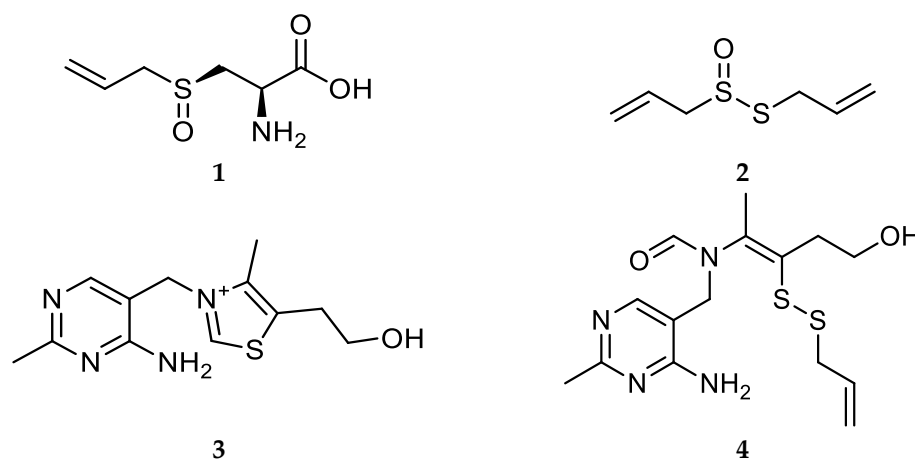


Figure 4. Chemical structures of alliin (1), allicin (2), thiamine (3), and allithiamine (4).

2.3. Grain Mixture

The grains were ground using a grinder (RT-02A; Rong Tsong Precision Technology Co., Ltd., Taichung, Taiwan) and mixed with thiamine powder (vitamin B₁ nitrate 100%, Bigsol, Berghain, Germany) in a 1:1 ratio. Afterward, 1 g of the mixture was suspended in water to achieve a 10% concentration extract; garlic was minced using a garlic press (KHC-1000G, Kitchen Art Co., Ltd., Shenzhen, China) and mixed at 150 rpm for 24 h. Subsequently, it was freeze-dried and used as a sample. The different grain mixture samples are *A. sativa* thiamine garlic (ASTG), *A. hypogaea* thiamine garlic (AHTG), *P. vulgaris* thiamine garlic (PVTG), and *R. nulubilis* thiamine garlic (RNTG).

2.4. Preparation of Garlic Samples and Standard Solutions for HPLC Analysis of Allicin

An ultrasonic bath was used to extract the mixture at 40 °C for 10 min. After extraction, the solution was centrifuged (10,000 rpm, 10 min, 4 °C) and filtered through a 0.20 μm polyvinylidene fluoride (PVDF) membrane filter to obtain the sample solution. The standard allicin (1 mg) was accurately weighed and dissolved in EtOH (1 mL) to prepare the stock solution. The solution was sonicated until complete dissolution and filtered through a 0.20 μm PVDF membrane filter. The stock solution was then appropriately diluted to prepare standard solutions with concentrations ranging from 0.015625 mg/mL to 0.25 mg/mL.

2.5. Preparation of Garlic Samples and Standard Solutions for HPLC Analysis of Alliin, Thiamine, and Allithiamine

The extraction solvent was prepared by mixing MeOH and water at an 80:20 (v/v) ratio and adjusting the pH to 3.0 with formic acid. The solutions were prepared by adding 1 mL

of extraction solvent to 5 mg of each sample, extracting at 40 °C for 10 min in an ultrasonic bath and filtering through a 0.20 µm PVDF membrane filter. The standard solutions were prepared by weighing 1 mg of two compounds, dissolving it in the extraction solvent, sonicating, filtering through a 0.20 µm PVDF membrane filter, and then diluting to achieve concentrations ranging from 1.0 to 0.0625 mg/g.

2.6. HPLC-UV Conditions of Allicin Analysis

The HPLC analysis was conducted using an Agilent 1260 Infinity II Quaternary Pump system equipped with a UV detector, following the methods described in previous studies [24]. Separation was achieved using a YMC Pack Pro C18 column (4.6 × 250 mm, 5 µm) maintained at 30 °C. The injection volume was set to 10 µL. The mobile phase consisted of an isocratic mixture of water containing 0.1% phosphoric acid (solvent A) and ACN (solvent B) at a ratio of 25:75. The flow rate was 1.0 mL/min. The analytes were detected at a wavelength of 208 nm (Figure 5). The mobile phase composition remained constant throughout the analysis, with solvents A and B maintained at 25% and 75%, respectively, for 30 min.

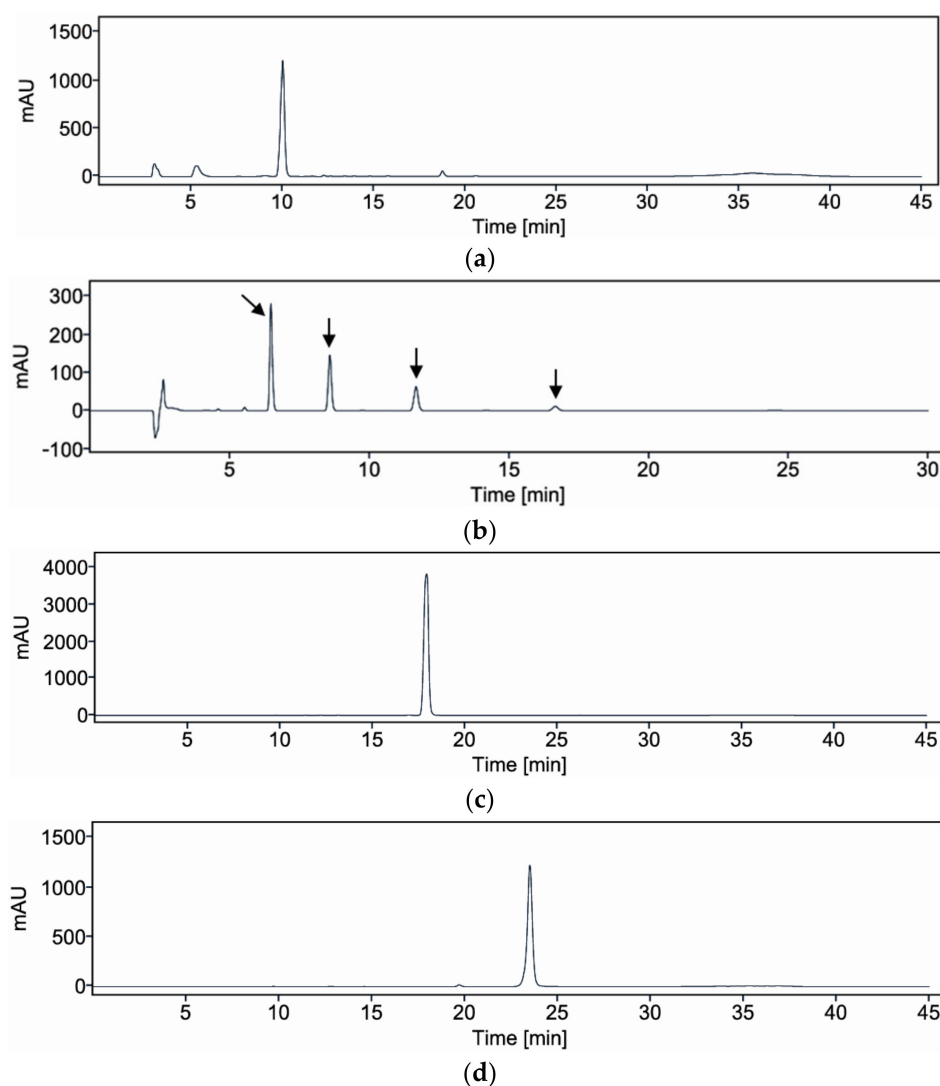


Figure 5. HPLC/UV chromatograms of alliin (a) allicin (DAS, 6.5 min; DAT, 8.6 min; DADS, 11.7 min; DATS, 16.6 min) (b), thiamine (c), and allithiamine (d).

2.7. HPLC-UV Conditions of Alliin, Thiamine, and Allithiamine Analysis

Reversed-phase HPLC analysis was performed on an Agilent 1260 Infinity II system equipped with a Quat Pump and UV detector, using an Aegispak C18-L column (3.0×150 mm, $5 \mu\text{m}$) at 30°C . A sample injection volume of $10 \mu\text{L}$ was used. The mobile phase consisted of 20 mM sodium dihydrogen phosphate monobasic and 10 mM 1-heptane sulfonic acid sodium salt in water, with the pH adjusted to 2.2 using phosphoric acid as solvent A and a mixture of ACN and solvent A (50:50) as solvent B for alliin and the MeOH as solvent B for thiamine and allithiamine. The flow rate was set to 0.3 mL/min . The detection wavelength was 208 nm for alliin and 254 nm for thiamine and allithiamine (Figure 5). The mobile phase gradient was programmed as follows: 0% B at 0 min, 30% B at 5 min, 54% B at 25 min, and 100% B from 30 to 32 min, followed by re-equilibration at 100% A from 37 to 45 min. Following previous reports, the analysis was performed under these conditions to ensure complete separation and detection of the target compounds [25,26].

2.8. Calibration Curves

Calibration curves were constructed to establish the relationship between the concentrations of standard compound solutions and the corresponding peak areas of each sample (Table 1). The linearity of the curves was assessed using the correlation coefficient (r^2). The sample concentrations were determined from the calibration curves. The calibration functions were defined in terms of the peak area (Y) and concentration (X, mg/mL), and the results are presented as means $\pm$ standard deviation (SD).

Table 1. Calibration curve equations for alliin (1), allicin (2), thiamine (3), and allithiamine (4).

Compound	t_R ¹	Calibration Equation ²	Correlation Factor, r^2 ³
alliin (1)	9.9	$Y = 18272.5X + 505.4$	0.9984
allicin (2)	6.5, 8.6, 11.7, 16.6	$Y = 15481.0X + 23.2$	1.0000
thiamine (3)	17.9	$Y = 68605.2X + 1081.1$	0.9971
allithiamine (4)	23.5	$Y = 23923.2X + 23.2$	1.0000

¹ t_R = retention time; ² Y = peak area, X = concentration of the standard (mg/mL); ³ r^2 = correlation coefficient for the five data points in the calibration curve.

2.9. Statistical Analysis

The HPLC analysis results are expressed as the mean $\pm$ SD derived from three independent experiments. Statistical analysis was performed using a one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. All statistical analyses and graphs were generated using GraphPad Prism 8.0.2 statistical software (GraphPad Software, Boston, MA, USA). NS means not significant, and *, **, ***, and **** are significant at $p < 0.05$, 0.01, 0.001, and 0.0001, respectively.

3. Results

3.1. Regional Variation in Alliin and Allicin Content

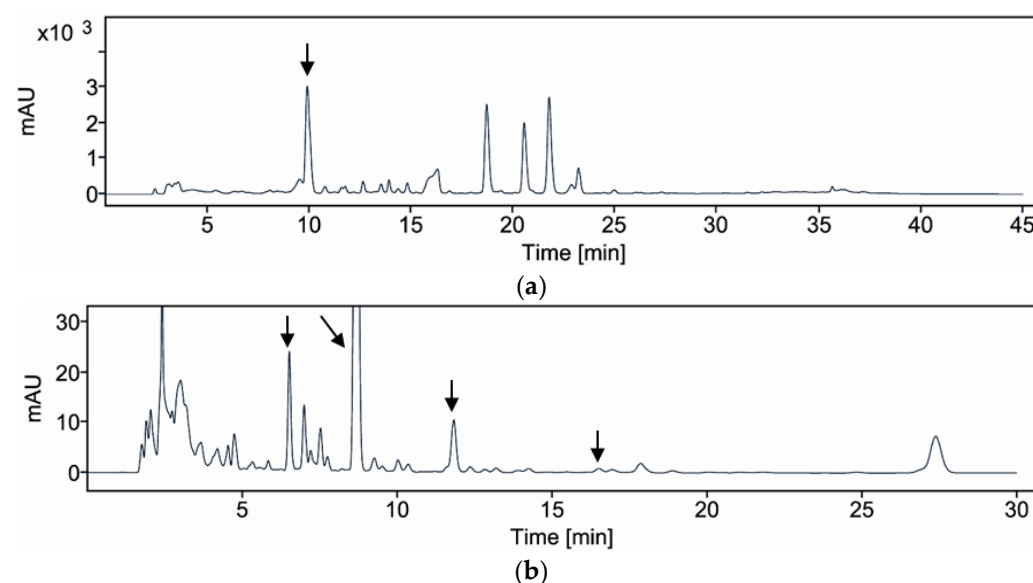
Garlic samples were collected from various regions of South Korea and analyzed to identify which had the highest allicin content to maximize the potential of garlic as a functional ingredient by targeting samples rich in sulfur-based bioactive compounds. The alliin and allicin content in these garlic samples varied significantly (Table 2 and Figure 6).

Jeju garlic had the highest allicin content (1.04 mg/g), making it the most promising candidate for functional food applications, despite having moderate alliin levels (19.47 mg/g). Uiseong garlic had the highest alliin content (24.90 mg/g) and relatively low allicin levels (0.12 mg/g).

Table 2. Alliin (1) and allicin (2) contents of *A. sativum* extract samples from different regions of South Korea.

Sample	Contents (mg/g) ****	
	Alliin	Allicin
Jeju garlic	19.47 ± 0.38 ^b	1.04 ± 0.01 ^a
Muan garlic	7.48 ± 0.01 ^e	0.06 ± 0.01 ^d
Namhae garlic	17.12 ± 0.40 ^c	0.28 ± 0.01 ^b
Uiseong garlic	24.90 ± 0.30 ^a	0.12 ± 0.01 ^c
Yeongcheon garlic	14.10 ± 0.01 ^d	0.26 ± 0.01 ^b

^{a–e} different letters indicate significant differences; **** means significant at $p < 0.0001$.

**Figure 6.** HPLC/UV chromatograms of Jeju garlic showing the peaks corresponding to alliin (9.9 min) (a) and allicin (DAS, 6.5 min; DAT, 8.6 min; DADS, 11.7 min; DATS, 16.6 min) (b).

3.2. Thiamine Contents of Raw Grains

The thiamine and allithiamine contents of the raw grain materials were also evaluated (Table 3). *R. nulubilis* was the only one exhibiting detectable thiamine levels (0.16 mg/g). Thiamine was not detected in *A. hypogaea*, *A. sativa*, or *P. vulgaris*. Trace amounts of thiamine alone did not directly correlate with the production of allithiamine in garlic–grain mixtures.

Table 3. Thiamine (3) content in grain raw materials powder samples.

Sample	Contents (mg/g) ****	
	Thiamine	Allithiamine
<i>A. hypogaea</i>	ND	ND
<i>A. sativa</i>	ND	ND
<i>P. vulgaris</i>	ND	ND
<i>R. nulubilis</i>	0.16 ± 0.01	ND

**** means significant at $p < 0.0001$; ND: not detected.

3.3. Thiamine and Allithiamine Contents in Garlic and Garlic–Grain Mixtures

Thiamine and allithiamine were first analyzed in garlic and grains alone. The result showed that thiamine is present only in trace amounts in the grains, whereas its content in the Jeju garlic was only 0.97 mg/g (Table 4). On the other hand, allithiamine was not detected in all grains and only trace amounts in the Jeju garlic sample. Due to these results, the garlic extract was mixed with the grains in order to investigate the effects of the grains when combined with garlic in the synthesis of allithiamine.

Table 4. Comparison of thiamine (3) and allithiamine (4) production between thiamine-free control samples and Jeju garlic–thiamine mixtures.

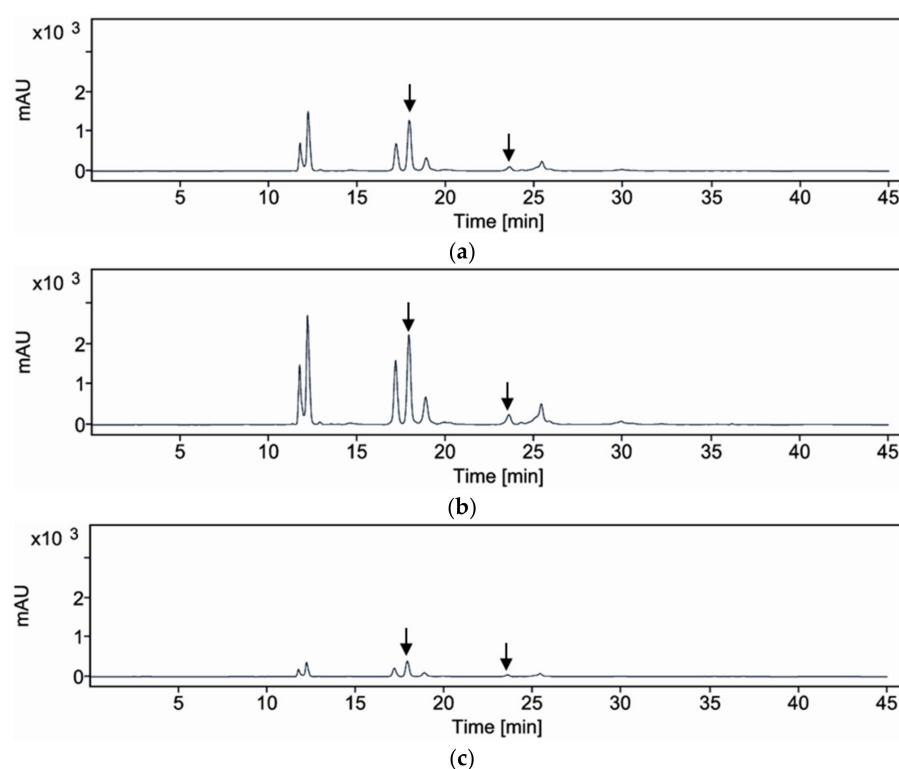
Control Sample	Contents (mg/g)	
	Thiamine	Allithiamine
AHG	tr	ND
ASG	tr	ND
PVG	tr	ND
RNG	tr	ND
Jeju + thiamine	0.97 ± 0.01	tr

tr: trace; ND: not detected.

The analysis of thiamine and allithiamine contents in garlic–grain mixtures revealed notable differences based on grain type (Table 5 and Figure 7). ASTG contained the highest levels of both thiamine (37.04 mg/g) and allithiamine (14.93 mg/g), making it the most effective mixture for allithiamine synthesis. AHTG and RNTG also showed a high level of allithiamine (6.37 and 6.92 mg/g), despite having undetectable thiamine levels in its raw form, indicating that grain matrix properties significantly influence the interaction between allicin and thiamine. Conversely, PVTG exhibited relatively low allithiamine levels (2.54 mg/g), suggesting that not all grains were equally effective in promoting allithiamine synthesis [27].

Table 5. Thiamine (3) and allithiamine (4) contents in grain raw materials, *A. sativum*, and thiamine mixture freeze-dried powder samples.

Sample	Contents (mg/g) ****	
	Thiamine	Allithiamine
AHTG	21.08 ± 0.01^b	6.37 ± 0.01^c
ASTG	37.04 ± 0.01^a	14.93 ± 0.01^a
PVTG	5.54 ± 0.01^d	2.54 ± 0.01^d
RNTG	16.06 ± 0.01^c	6.92 ± 0.01^b

a–d different letters indicate significant differences; **** means significant at $p < 0.0001$.**Figure 7.** Cont.

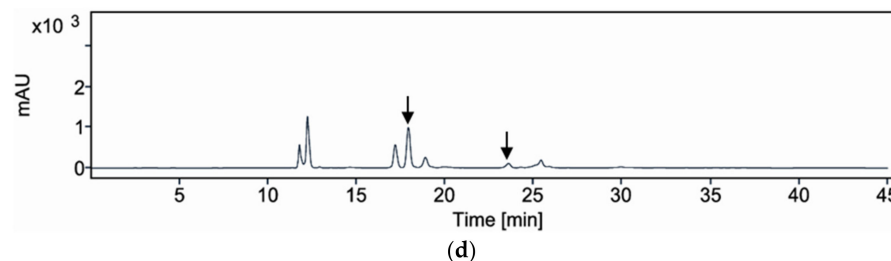


Figure 7. HPLC/UV chromatograms of thiamine (17.9 min) and allithiamine (23.5 min) of ASTG (a), AHTG (b), PVTG (c), and RNTG (d).

4. Discussion

In this study, garlic sourced from different regions of South Korea was analyzed for its allicin and alliin content. The results showed varying content in these garlic extracts. These differences suggest that factors other than alliin concentration, such as alliinase activity and environmental conditions, determine the allicin levels. Previous studies have shown that alliinase activity varies depending on the garlic cultivation environment, including soil composition, climate, and agricultural practices [28], likely due to the differences in enzyme activity and substrate accessibility. Specifically, in intact cells, alliin and alliinase are separated, but cutting or crushing releases them, enabling interaction [29]. High substrate concentration and effective diffusion enhance activity, while limited solubility, inhibitors, or dense tissues reduce it. Optimal conditions like proper pH, hydration, and temperature also improve substrate binding and enzyme efficiency.

A previous study analyzed the physiological activities of garlic extracts from eight different cultivating areas (three areas from Daejeong and four major cultivating areas from Danyang, Uiseong, Taean, and Namhae) in Korea and one from China. Their results showed that Muan garlic exhibited the lowest allicin content (0.06 mg/g), with Yeongcheon and Namhae garlic samples showing intermediate levels ranging from 0.26 to 0.28 mg/g. These results are similar to previous studies that showed that the allicin content of garlic varies considerably by region [30]. Danyang garlic showed the highest allicin content at 4.57 mg/g, followed by Uiseong (4.22 mg/g), Jeju (3.34 mg/g), and Namhae (3.28 mg/g). These differences likely reflect regional variations in cultivation conditions, such as soil properties, climate, and agricultural practices [31]. These differences can be attributed to environmental factors like UV light, soil type, and even the amount of rain the region the garlic was sourced from receives [32]. A recent study analyzed the bioactive compounds from different varieties of garlic from different regions [33]. They discovered a link between food composition and environmental conditions, which can be utilized to identify garlic according to its origin.

To investigate the allithiamine synthesis in garlic and garlic–grain mixtures, Jeju garlic was combined with different grains. These grains are known to harbor thiamine, a compound needed to synthesize allithiamine when reacted with allicin. However, when the thiamine content of the grains was measured, there was little to none detected in the grain samples. Hence, the grains were supplied with thiamine to compensate for the lack thereof. The addition of thiamine powder in the grains is a usual practice around the world called fortification, wherein the degraded or lost thiamine during the processing is augmented [34]. By adding thiamine powder, the losses are compensated. Interestingly, although *R. nulubilis* was the only grain with detectable thiamine content in its raw state, the garlic mixture (RNTG) did not yield the highest allithiamine content. This observation highlights the importance of the structural and chemical properties of the grain matrix, rather than the initial thiamine content, in facilitating the reactions between allicin and thiamine.

The choice of Jeju garlic as the base for the mixtures was based on its high alliin content, which was the highest among all examined samples. Since alliin plays a crucial role in the synthesis of allithiamine through its reaction with thiamine, Jeju garlic was determined to be the most suitable candidate for optimizing allithiamine production. This aligns with this study's objective of leveraging the enhanced bioavailability of allithiamine compared to water-soluble thiamine. The biosynthetic pathway of allithiamine involves the reaction of thiamine with alliin, wherein the sulfur groups in alliin interact with the reactive centers of thiamine to form disulfide bonds, such as thiamine-allyl disulfide (Figure 1). However, the efficiency of this reaction varies depending on the physical and chemical properties of the grain matrix. Previous studies have shown that the grain matrix composition can significantly influence the diffusion of specific compounds in various ways [35–37]. Additionally, the enzymatic activity of alliinase, which converts alliin to alliin, is influenced by environmental factors such as pH, temperature, and substrate accessibility [38]. Although no significant differences in enzymatic activity between garlic samples were observed in this study [38], variations in the reaction conditions within the grain matrices could still influence allithiamine production.

Alliin, a key sulfur compound in garlic, readily interacts with thiol-containing proteins and enzymes to form disulfide bonds [39,40]. Although these interactions are not specific to grains, they highlight alliin's general chemical reactivity. Thiamine, meanwhile, is prone to degradation by thiaminases—enzymes typically found in certain raw or fermented foods, such as fish and shellfish, but not commonly associated with grains. The absence of thiaminases in the grains ensured that the stability of thiamine and its derivatives was maintained in these mixtures [41].

The findings of this study emphasize the importance of grain selection for optimizing the synthesis of allithiamine in garlic–thiamine mixtures. The high allithiamine levels observed in ASTG demonstrate its potential as a functional food candidate with enhanced bioavailability of bioactive compounds. In contrast, the relatively low allithiamine levels in PVTG highlight the need to carefully consider grain properties when designing such formulations carefully. These results provide valuable insights for developing functional foods to improve metabolic health through improved thiamine availability. However, the mechanisms behind them are not yet clear or have not been reported by previous work, particularly how the grains aided in the production of allithiamine when mixed with garlic. Specifically, elucidating these mechanisms and exploring the different ratios of grain and garlic mixtures can be explored as well as optimizing allithiamine synthesis. In addition, the variability of the allithiamine according to the temperature and pH set during the mixing was not tackled in this work, providing an avenue for further studies in this area. The influence of the enzymes involved should also be elucidated. Scientists can improve the allithiamine synthesis efficiency, scale up industrial production, and investigate structural modifications to boost allithiamine stability and efficacy. Ultimately, this study highlights the significant influence of garlic variety and grain type on allithiamine synthesis and offers practical guidelines for developing functional foods. Future research should investigate additional garlic–grain combinations and explore the biochemical mechanisms driving these interactions to enhance the health benefits of these mixtures further.

5. Conclusions

This study illustrates the importance of garlic variety and the grain matrix on allithiamine production, a lipid-soluble thiamine derivative with increased bioavailability. Jeju garlic, with the highest alliin content, was identified as the most promising source of sulfur-based bioactive uses. Grain selection had a considerable impact on allithiamine production, with ASTG generating the greatest quantities despite containing no detectable

thiamine in its raw form. These findings emphasize the significance of matrix-specific variables, such as physical and chemical features, over initial thiamine levels. However, the specific properties of the samples as well as the conditions of mixing should be optimized, as these are just arbitrary in the present work. This research lays the groundwork for the development of functional foods aimed at improving thiamine bioavailability and metabolic health. The results from this study provide a lot of facets for further investigations due to the gaps they have presented. Hence, future research should improve reaction conditions and investigate novel garlic–grain combinations to increase allithiamine production and medicinal potential.

Author Contributions: HPLC analysis and writing, C.-D.L.; alliin analysis, J.C.; allicin analysis, M.K.; thiamine analysis, M.J.; allithiamine analysis, E.Y.; resources and experimental design, J.K. and S.L.; supervision, writing, and editing, S.L. All authors have read and agreed to the published version of the manuscript.

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