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Effect of Yogurt on the Deodorization of Raw Garlic (*Allium sativum* L.) Sulfur Volatiles in Breath and the Roles of Its Components

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Abstract: Consumption of garlic leads to the persistence of "garlic breath" due to the presence of malodorous sulfur volatiles which may persist for as long as 24 h. Therefore, the purpose of this study is to investigate the effect of yogurt and its components on the deodorization of garlic sulfur volatiles in breath and study the roles of these components in deodorization. Raw garlic was consumed with different treatments and at different times for breath analysis. Different components were mixed with the garlic for headspace analysis. Volatiles were measured using selected-ion flow-tube mass spectrometry. Consuming yogurt at the same time as garlic was more effective than consuming it before or after. Yogurt was the most effective at deodorization, followed by the emulsion, then protein or fat alone. Decreasing the pH of protein solutions increased deodorization because changes to the structure of the proteins exposed more binding sites for the volatiles, while decreasing the pH of water or fat had no effect on deodorization. Whey protein deodorized better than casein due to the presence of more cysteine binding sites for volatiles. This study proposes that the fat, protein, microbial culture, and water in yogurt have synergistic effects on the deodorization of garlic volatiles. This study's findings can help in the development of novel products targeting sulfur volatiles, with broad applications for mitigating malodors produced by garlic.

Keywords: garlic; deodorization; yogurt; fat; protein; microbes; water; pH; emulsions; synergism

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1. Introduction

Garlic (*Allium sativum* L.) is a popular condiment known for its flavor-enhancing properties. Consumption of garlic leads to the persistence of garlic breath due to the presence of malodorous sulfur volatiles which may persist for as long as 24 h [1,2]. The sulfur volatiles allyl mercaptan, allyl methyl sulfide, allyl methyl disulfide, diallyl disulfide, and methyl mercaptan are responsible for bad odor [3–7]. These volatiles are formed when garlic is crushed or chopped [8]. When raw garlic is consumed in its crushed form, allicin is created by the action of CS-lyase (alliinase) on alliin [9–11]. However, in vivo formation of these volatiles also continues inside the body. As raw garlic is ingested, allicin undergoes a cascade of conversions, including breakdown in the stomach to diallyl disulfide and allyl methyl disulfide by glutathione enzyme [12]. Further, the allicin or diallyl disulfide transforms to allyl mercaptan in the blood cells via S-allylmercaptocysteine [13]. When paired with S-adenosylmethionine, allyl mercaptan is subsequently converted to allyl methyl sulfide by the enzyme methyltransferase [13].

A diverse array of foods, including vegetables, fruits, and herbs, have been employed for the purpose of mitigating malodorous garlic breath. Studies have shown the effects of treatments such as mint leaves (both spearmint and peppermint), pear, loquat, peach, plum, prune, apricot, cherry, grape, chicory, udo, perilla, burdock, potato, eggplant, dried and fresh herbs, lemon juice, green tea, spinach, parsley, basil, kiwi, raw egg, boiled rice, mushrooms, apple, raw lettuce, milk, and oil on the deodorization of garlic breath [14–20].

While several foods have shown breath deodorization potential, the role of yogurt and the interaction of its components remains unexplored in vivo. In vitro, yogurt produced a significant reduction in garlic sulfur volatiles in a headspace study [21]. The major components of yogurt are water, fat, proteins, and yogurt culture. Separately, fat, dairy proteins, and water have been shown to reduce the concentration of garlic sulfur volatiles [20,21]. Fat binds garlic sulfur volatiles through hydrophobic interactions [21,22]. Many proteins interact with sulfur volatiles through covalent and non-covalent interactions. Caseins interact through non-covalent reactions and covalent redox interactions with allicin and diallyl disulfide [23–26]. Whey protein, i.e., beta-lactoglobulin, alpha-lactalbumin, and albumins, interacts with diallyl disulfide and allyl mercaptan thorough non-covalent interactions [27–29]. Water binds through portioning the garlic sulfur volatiles into different phases [20,21]. The microorganisms in yogurt have not been tested for deodorization of garlic, but studies have shown that the prebiotic cultures used in yogurt reduce halitosis, including volatiles such as hydrogen sulfide, dimethyl sulfide, and methyl mercaptan [30–32]. Compared to existing methods for deodorization of garlic sulfur volatiles in breath, such as chewing gums or chemical breath fresheners, yogurt may offer a natural and widely available alternative. Additionally, its potential health benefits make it a preferable choice for consumers seeking healthier options. Therefore, this study aims to fill this gap by examining the effectiveness of yogurt in deodorizing garlic breath. The purpose of this study is to introduce an innovative approach by investigating yogurt and its components for their potential to deodorize garlic sulfur volatiles in breath. The study will also delve into understanding the role of these components and their interactions, including fat, proteins, yogurt microorganisms, water, emulsions, and pH, to propose the mechanisms behind the deodorization of garlic sulfur volatiles by yogurt.

2. Materials and Methods

2.1. Garlic and Treatments Preparation

Garlic was peeled by hand, then cut into slices of approximately 1 mm thickness. Different treatments, such as whole Greek yogurt (Kroger, Columbus, OH, USA), water, 5% butter fat (Kroger, Columbus, OH, USA), 9% different dairy proteins (whey protein concentrate, whey protein isolate, micellar casein, calcium caseinate, milk protein concentrate, and milk protein isolate (Mill Haven Foods, New Lisbon, WI, USA)), fat–protein emulsion, and microbes (*L. acidophilus* and *B. bifidum*) were used. For proteins, 9 g of protein was mixed with 100 g of water and rehydrated by mixing for 4 h with a magnetic stirrer. For fat, 5 g of butter fat was mixed with 100 g water at 50 °C for 7 to 10 min using a magnetic stirrer, and cooled to room temperature before use. To determine the effects of the pH levels of water, fat, and different proteins, lactic acid and sodium hydroxide were used to adjust the pH to 4.4 and 7 for all the treatments. For emulsions, 5% butter fat was mixed with whey protein concentrate or milk protein concentrate. For microbes, 9% non-fat milk powder was mixed with water. The milk sample was pasteurized at 85 °C for 20 min. Then, the milk was cooled to 40–41 °C. One tablespoon of yogurt was added to 100 g milk as the starter culture. A quantity of 10% of sucrose was added to the milk mixture to optimize the growth of lactic-acid bacteria. After inoculation, the milk was incubated at 41 °C for 4 h to maintain favorable conditions for the growth of microorganisms. A total of 6 g of garlic was mixed with 100 g of milk and milk inoculated with yogurt culture, left for 30 min, and analyzed. The control was 6 g garlic.

2.2. Selected-Ion Flow-Tube Mass Spectrometry (SIFT-MS) Breath Analysis

For breath analysis, six grams of chopped garlic was chewed for 60 s and then swallowed (control). Quantities of 100 g of different treatments were consumed along with the 6 g of garlic, and mixed and chewed in the mouth for 60 s before swallowing. The treatments were whole fat Greek yogurt, 5% butter fat, 9% whey protein concentrate, and water. To determine the effect of time of consumption, yogurt was consumed with garlic 5 min before or 5 min after the consumption of garlic. The volatile levels in the breath were measured with selected-ion flow-tube mass-spectrometry (SIFT-MS). Breath analysis was performed using 5.08 cm straws (Red Slim Straws; Dixie Consumer Products LLC., Atlanta, GA, USA). The breath sample was provided by placing the mouth on one end of the straw and other end of the straw around SIFT-MS's passivated needle. To prevent air escape, the straw was placed tightly around the passivated needle attached to SIFT-MS, encircling it completely. The volatiles in the breath were measured in 120 s scans, beginning immediately after chewing. Breath-scan patterns of 15 s exhales and 5 s inhales were used to measure the span of the breath. Every breath scan had six distinct breaths. SIFT-MS breath measurements were taken at 0, 5, 10, 20, 30, 40, 50, and 60 min after garlic consumption. The same technique was used with different treatments. Exhalation of breath was indicated by acetone levels in the breath. For each exhale, the time when acetone was within 10% of its maximum level was considered the precise timing for each exhale. The average concentration of each volatile for this time period was calculated by Time Series Modelling. The background was subtracted to obtain the final concentration. For each treatment, three replicates were performed. Table 1 lists the volatiles that were tested.

Table 1. Properties of volatiles tested in selected-ion flow-tube mass spectrometry (SIFT-MS) headspace and breath analysis.

Volatile Compound	Ion Product	Reagent Ion	*m*/*z*	Reaction Rate (k) 10^{-9} cm^3 s^{-1}
Allyl mercaptan	C_3H_6S	NO^+	74	2.4
	$C_3H_6S.H^+$	H_3O^+	75	2.6
Allyl methyl disulfide	$C_4H_8S_2$	NO^+	120	2.4
	$C_4H_8S_2.H^+$	H_3O^+	121	2.6
Allyl methyl sulfide	$C_4H_8S^+$	NO^+	88	2.5
	$C_4H_8S.H^+$	H_3O^+	89	3
Diallyl disulfide	$(C_3H_5)_2S_2.H^+$	H_3O^+	147	3
Methyl mercaptan	$CH_4S.H^+$	H_3O^+	49	1.8

The experiment was conducted on a single subject (Figure 1). The subject was a 28-year-old female with no significant history of chronic diseases that could affect the study's results. The subject did not smoke and had no known digestive disease. To ensure that the effects observed were solely due to garlic consumption, the participant adhered to a specific regimen. The subject consumed their last meal in the evening and abstained from eating overnight for at least 12 h before consuming the garlic. In the morning, the participant cleaned their mouth using toothpaste and mouthwash, ensuring a gap of at least 2 h before the ingestion of garlic. Additionally, the subject's breath was tested before consuming garlic, ensuring that any changes in breath volatiles could be attributed specifically to the garlic consumption.

2.3. Selected-Ion Flow-Tube Mass Spectrometry (SIFT-MS) Headspace Analysis

A quantity of 100 mL of each treatment was mixed with 6 g of raw garlic. A 500 mL Pyrex bottle, capped with PTFE (polytetrafluoroethylene) with a silicone septa cap was used to hold the sample. The sample was held at room temperature for 30 min. SIFT-MS headspace analysis was performed using an SIM (selected ion mode) scan at 20 °C ± 2. The standard gases octafluorobenzene, hexafluorobenzene, tetrafluorobenzene, ethylene, isobutane, toluene, and benzene were used to calibrate the SIFT-MS. The 18-gauge passivated needle connected to the inlet of a SIFT-MS machine (SYFT Voice200ultra, Syft Ltd., Christchurch, New Zealand) punctured the silicone septa cap of Pyrex bottle in which the sample was placed to sample the volatiles from the headspace. The volatiles were analyzed using Syft VOICE-200 software (v.1.4.9.17754, Syft Technologies Ltd., Christchurch, New Zealand). Before testing the samples, an empty 500 mL Pyrex bottle was used as a blank.

For each treatment, three replicates were performed. Each headspace reading took 95 s. Table 1 lists the volatiles that were tested.

Figure 1. Breath (in vivo) and headspace (in vitro) analysis of garlic sulfur volatiles using SIFT-MS to determine the effect of yogurt on sulfur garlic volatiles.

2.4. Data Analysis

JMP® Pro version 16.0.0 (512340) (Statistical Discovery, Cary, NC, USA) was used for the analysis of data obtained from SIFT-MS. Three replicates were used for each treatment. One-way or two-way analysis of variance (ANOVA) was performed to analyze data, and Fisher's least significance difference (LSD) or the Games–Howell test was used for comparison of all pairs.

3. Results and Discussion

3.1. Effect of Yogurt and Its Components on the Deodorization of Garlic Breath

To determine if yogurt and its components can deodorize garlic breath, yogurt, 5% butter fat, 9% whey protein concentrate, or water was consumed with the garlic. The concentrations of all sulfur-based volatiles were significantly reduced in the breath after consuming yogurt, fat, protein or water (Figure 2 and Tables A1–A5). All of the volatiles showed similar deodorization trends, so only diallyl disulfide, allyl mercaptan, and allyl methyl sulfide are shown in Figure 2.

Yogurt quickly deodorized the volatiles to below their odor detection thresholds (ODTs), within 10 min for diallyl disulfide (ODT 4.30 ppb) [33] and allyl methyl disulfide (ODT 0.30 ppb) [33], and within 60 min for allyl mercaptan (ODT 0.05 ppb) [34] and methyl mercaptan (ODT 0.66 ppb) [35]. Diallyl disulfide, allyl methyl disulfide, allyl mercaptan, and methyl mercaptan are responsible for the malodor in the mouth and metabolize quickly inside the body [4,5,19,20,36–38]. The interactions of these volatiles with yogurt in the mouth and their further degradation inside the body result in rapid deodorization of these garlic volatiles. However, after 60 min, allyl methyl sulfide (ODT 0.61 ppb) [33] was still above its odor detection threshold. There are two reasons allyl methyl sulfide takes longer to deodorize. First, it continues to be formed inside the body for several hours both due to methylation of allyl mercaptan and diallyl disulfide by S-adenosylmethionine in the blood, and due to its formation in the gut by microflora [39]. Second, unlike the other sulfur volatiles, allyl methyl sulfide is slow to be metabolized [5]. After its formation in the gut, allyl methyl sulfide moves into the blood and then into the lungs, from whence it is exhaled out through the breath. Eventually it is metabolized into allylmethylsulfoxide and allylmethylsulfone, which are odorless [40].

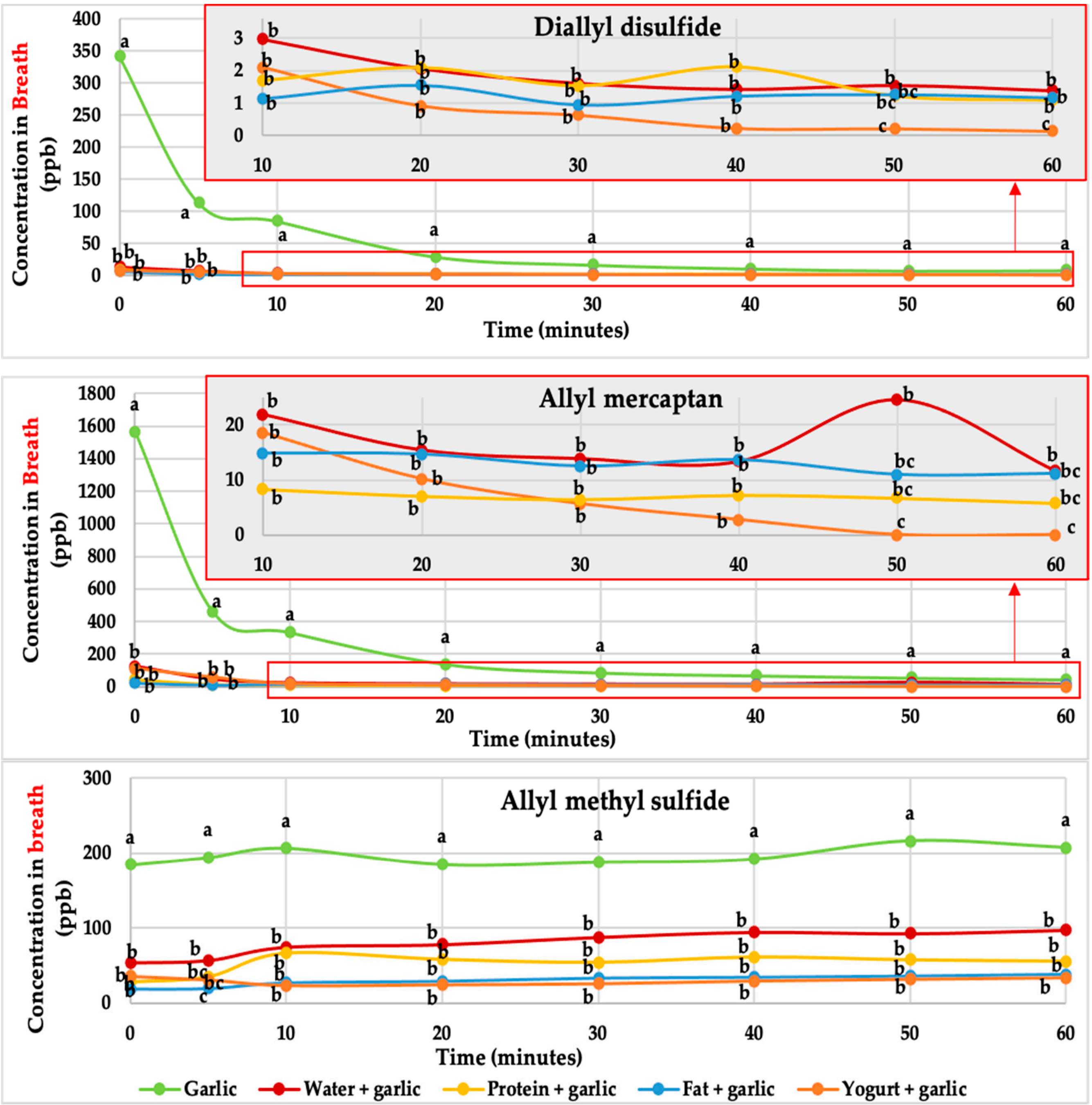

Figure 2. Effects of water, 9% protein, 5% butterfat, and yogurt on deodorization of garlic sulfur volatiles in breath. Treatments within the same time interval with different letters are significantly different ($p < 0.05$).

Yogurt was a more effective deodorizer than were its individual components (Figure 2). When individual components are compared, fat and protein were more effective than water in deodorization of garlic volatiles (Tables A1–A5). When raw garlic is chewed, it induces painful burning and prickling sensations [41], because sulfur volatiles activate perivascular sensory nerve endings in the mouth [42]. In this study, it was observed that ingesting fat significantly reduced the painful sensations, followed in efficacy by the protein solution, while water was the least effective. This further confirms that these treatments are binding the sulfur volatiles inside the mouth.

3.2. *Effect of Time of Consumption on the Deodorization of Garlic Breath*

To determine if the timing of yogurt consumption affects deodorization of garlic sulfur volatiles, yogurt was consumed before, with, or after garlic. All methods of consumption significantly reduced the concentration of all the garlic sulfur volatiles. However, the most effective method for deodorizing garlic breath was the simultaneous consumption of yogurt with garlic, followed by consuming yogurt before garlic, then consuming yogurt after garlic (Figure 3 and Tables A6–A10).

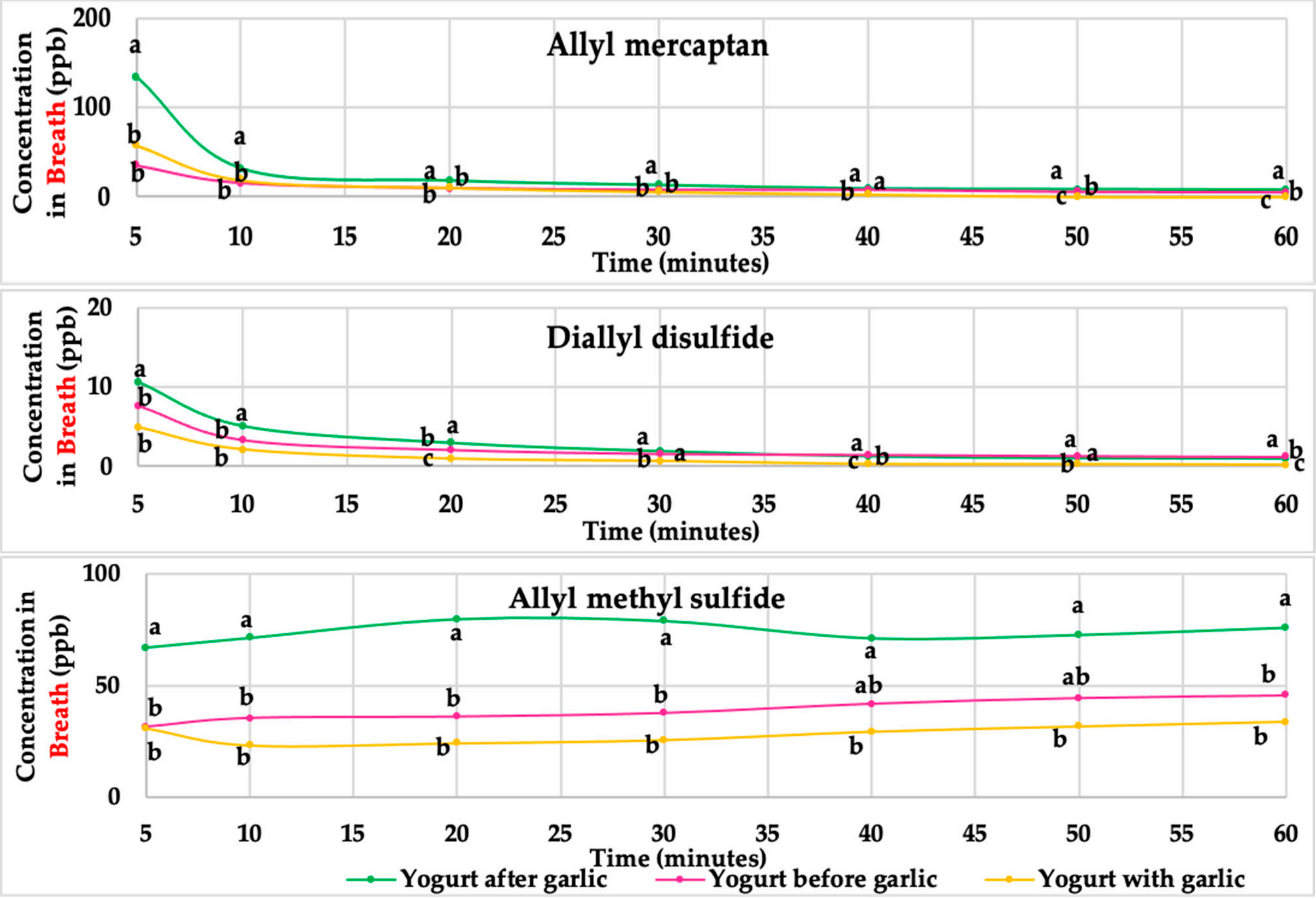

Figure 3. Effect of time of yogurt consumption on deodorization of garlic sulfur volatiles in breath. Treatments within the same time interval with different letters are significantly different ($p < 0.05$).

When yogurt is consumed simultaneously with garlic, the interaction of yogurt components (such as fat, protein, and water) with the garlic volatiles is initiated in the mouth and continues inside the body, resulting in rapid deodorization. Consuming yogurt after garlic produces the slowest deodorization, because the volatiles form inside the mouth and deodorization does not begin until the yogurt and garlic mix in the stomach. Similarly, others have reported that when milk was added to garlic before consumption, it resulted in greater deodorization than did consuming milk after ingesting garlic [20]. Interestingly, consuming yogurt before garlic provided some advantage over consuming the yogurt after the garlic. This indicates that the yogurt coats the inside of the mouth, and that the yogurt residue interacts with the garlic in the mouth. Thus, reducing the sulfur volatiles initially in the mouth, before they enter the body, results in decreased production of all sulfur volatiles. This is especially true for allyl methyl sulfide, which is the volatile that continues to form inside the body, persisting for more than 24 h. Thus, consumption of yogurt with, or possibly before, food containing garlic should lead to the greatest reduction in garlic breath.

3.3. The Role of the Components of Yogurt in Deodorization of Garlic Sulfur Volatiles in Headspace

To better understand the effect of individual components on deodorization, further studies were conducted in vitro. Yogurt and its individual components, i.e., water, protein, fat, and the fat–protein emulsion, were mixed with garlic (Figure 4). All of the treatments effectively deodorized the garlic sulfur volatiles (90–99%). Yogurt was the most effective treatment, followed by its individual components. Due to the high concentration of sulfur volatiles in garlic (control), the differences between the treatments cannot be seen in Figure 4. In order to see the effects of the different treatments on garlic sulfur volatiles, the garlic values are excluded from all subsequent graphs in this paper.

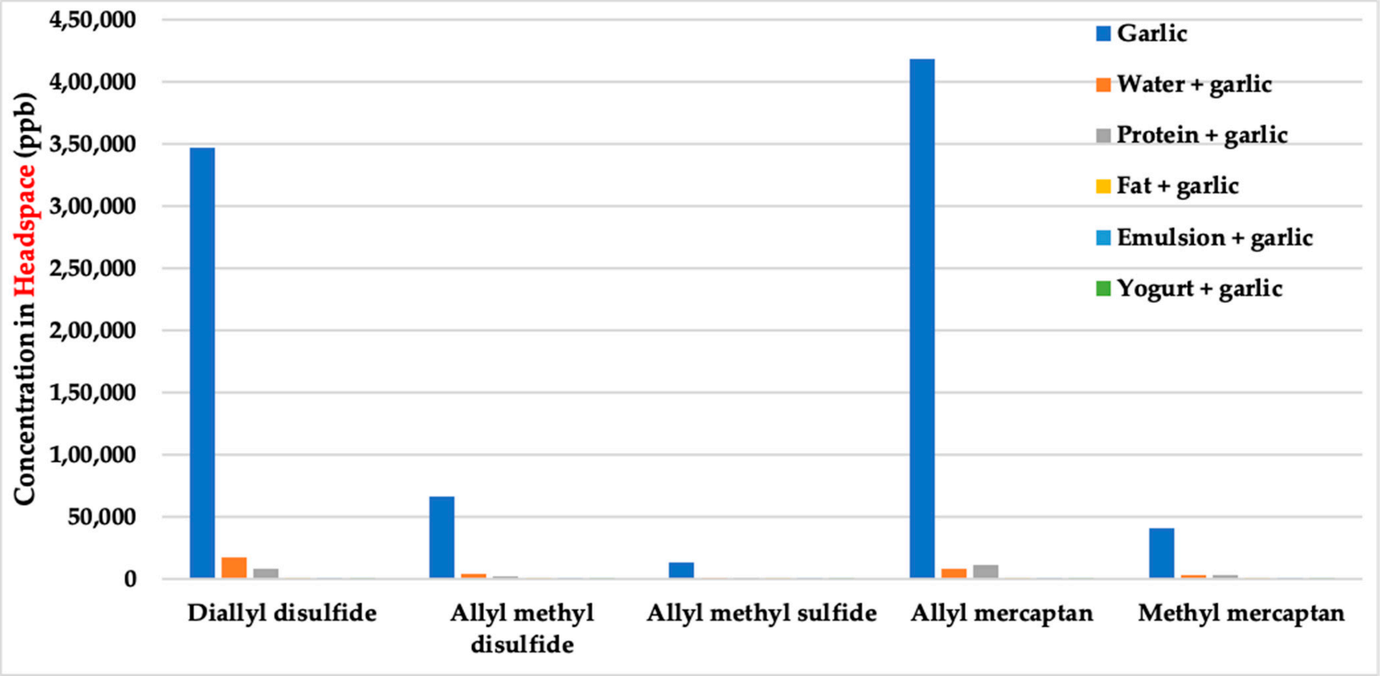

Figure 4. Effects of yogurt and its components on deodorization of garlic sulfur volatiles.

3.3.1. Effects of Protein pH and Type on the Deodorization of Garlic Sulfur Volatiles

Protein was one of the components of yogurt which produced significant deodorization (Figure 5). The ability of different dairy proteins to deodorize garlic sulfur volatiles when pH is adjusted to neutral (pH 7) or to below the isoelectric point of the protein (pH 4.4) was tested by mixing garlic with six forms of dairy protein: whey protein concentrate (WPC), whey protein isolate (WPI), micellar casein (MC), calcium caseinate (CC), milk protein concentrate (MPC), and milk protein isolate (MPI). All proteins greatly (90–99%) deodorized garlic sulfur volatiles at both pH levels (Table A11).

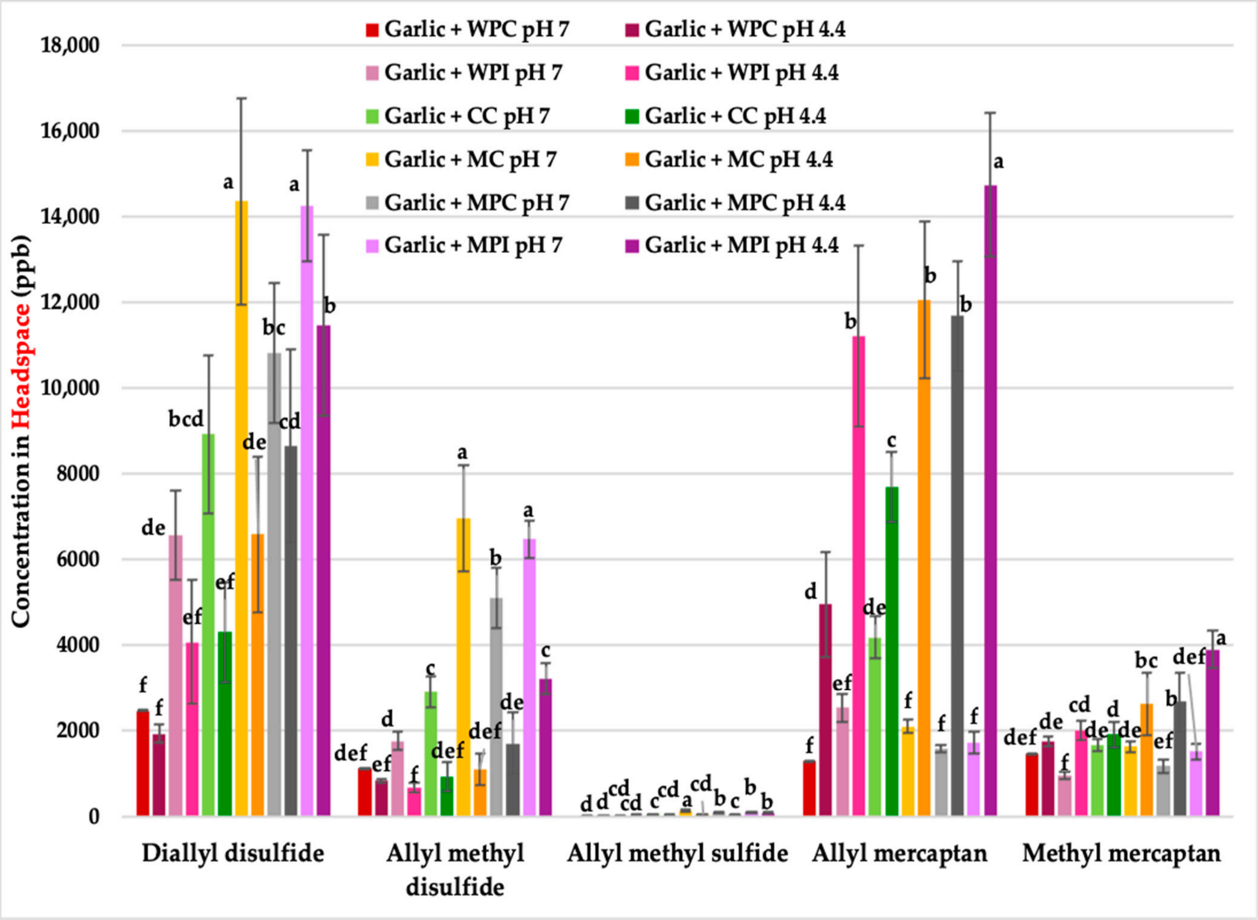

Figure 5. Effect of protein pH (4.4 and 7) and type on deodorization of garlic sulfur volatiles. WPC—whey protein concentrate; WPI—whey protein isolate; CC—calcium caseinate; MC—micellar casein; MPC—milk protein concentrate; MPI—milk protein isolate. Treatments within the same volatile with different letters are significantly different ($p < 0.05$).

Effects of Protein Type and Form on the Deodorization of Garlic Sulfur Volatiles

Overall, whey protein was the most effective deodorizer, followed by casein and milk protein (Figure 5 and Table A11). Whey protein contains 87% more cysteine than does casein [43]. The higher cysteine content in whey protein is most likely responsible for the higher deodorization of sulfur volatiles by whey. Milk protein consists of 80% casein and 20% whey proteins [44], and thus contains 50% more cysteine than casein [43]. At first, it would seem logical that milk protein would have produced greater deodorization than casein due to its higher cysteine content. However, in milk protein, some of the whey protein is bound to the K-casein in the casein micelles by disulfide bonds and hydrophobic interactions [29,45]. We hypothesize that this whey–casein interaction decreases the availability of the cysteine binding sites for the sulfur volatiles, thus decreasing the deodorization ability of whey–casein compared to whey alone. It has previously been reported that at neutral pH, whey protein is the most effective deodorizer, followed by casein and milk protein [21].

Concentrates were generally more effective than isolates, highlighting the importance of protein form in the deodorization process (Figure 5 and Table A11). The more extensive processing of isolates leads to more protein–protein interaction [46,47] which may result in fewer free thiol groups in cysteine being available to bind garlic sulfur volatiles, and thus less deodorization of sulfur volatiles.

Calcium caseinate generally produced greater deodorization than micellar casein (Figure 5 and Table A11). Although both calcium caseinate and micellar casein contain the same total amount of cysteine [43], in micellar casein, the caseins are bound together [48]. The cysteines in the alpha S2 casein are buried inside the structure and unavailable, and only the cysteines in the kappa casein are available to bind the sulfur volatiles. In contrast, in calcium caseinate, the cysteines in both alpha S2 and kappa casein are available to bind volatiles.

Effects of Protein pH on the Deodorization of Garlic Sulfur Volatiles

While the proteins remain in the same order of deodorization effectiveness at both pH conditions, under acidic conditions (pH 4.4), the effects on specific volatiles are more complicated. The volatiles can be divided into disulfides, mercaptans, and sulfides. Proteins such as whey and casein bind garlic sulfur volatiles through interactions such as disulfide–sulfhydryl interchange reactions and disulfide linkages [23–26,28,49,50], which results in deodorization of these volatiles. The disulfide volatiles bind with proteins through disulfide–sulfhydryl interchange reactions, while the mercaptans bind through disulfide linkages. The sulfides are oxidized into odorless byproducts by an unknown reaction.

For the disulfide volatiles, the deodorizing ability of the proteins was significantly increased by lowered pH (Figure 5). As the pH is decreased to 4.4, which is below the isoelectric points of both casein and whey, the solubility of the protein decreases, and hydrophobic interactions increase. Thus, at pH 4.4, proteins interacted more strongly with the hydrophobic disulfide volatiles, diallyl disulfide and allyl methyl disulfide, than when at neutral pH, creating greater deodorization.

Interestingly, while most of the disulfides are bound, some of the disulfides are instead converted to mercaptans through the disulfide–sulfhydryl exchange reaction by the action of cysteine [23–26,28,49–51]. The disulfide–sulfhydryl exchange reaction between diallyl disulfide and the protein results in the formation of one free allyl mercaptan and one allyl mercaptan bound to the protein (Figure 6).

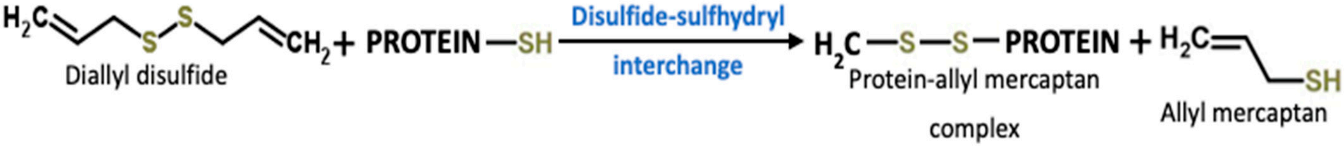

Figure 6. Formation of protein-allyl mercaptan and free allyl mercaptan complex from diallyl disulfide.

Based on the structure of allyl methyl disulfide, we theorize that it similarly undergoes a disulfide–sulfhydryl exchange reaction with the cysteine, resulting in the formation of one free allyl mercaptan and one methyl mercaptan bound to the protein (Figure 7). During this reaction, sulfhydryl exchange occurs between the S-S bond in the disulfide volatile and the free thiol group in the side chain of cysteine in the protein. This leads to the formation of a disulfide linkage between the protein and one of the formed mercaptans while the other mercaptan is released (Figures 6 and 7). This conversion of disulfides to mercaptans is faster at pH 4.4 than when at pH 7 due to the faster rate of the thiol–disulfide interchange reaction at pH 4.4. The thiol–disulfide interchange reaction involves the nucleophilic attack of the thiol group of cysteine on the S-S bond of the disulfide volatiles [52]. At pH 7, around 89% of the cysteine is protonated and present as thiols, but as the pH decreases, more of the thiol groups protonate to form thiols [53–55]. The increase in thiol concentration at pH 4.4 means more thiol–disulfide interchange, which results in a lower concentration of disulfides, but a higher concentration of mercaptans at pH 4.4 than at pH 7, as seen in Figure 5.

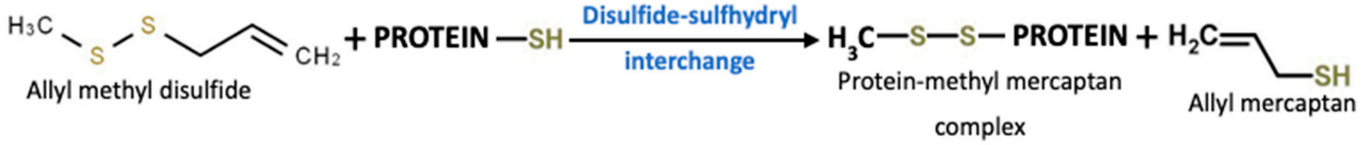

Figure 7. Formation of protein-methyl mercaptan complex and free allyl mercaptan from allyl methyl disulfide.

Effects of pH–Protein Interaction on the Deodorization of Garlic Sulfur Volatiles

In whey protein, as the pH decreased, the increase in the binding of the disulfide volatiles was not significant, because the binding sites were already exposed due to the heat denaturation and unfolding that occurred during the production of whey protein concentrate and isolate [56,57]. Thus, the disulfide concentration was unaffected by pH, and the mercaptan concentration increased at low pH, as already explained.

However, in casein and milk protein, lowering the pH increased the binding of disulfide. As the pH is lowered to 4.4, below the isoelectric point of casein, the calcium phosphate holding the micelles together solubilizes and the caseins dissociate [58–60]. Alpha S2 which was hidden in the micelle core is now available to interact with the sulfur volatiles, in addition to the kappa casein, increasing disulfide binding.

3.3.2. Effects of Water and Fat pH on the Deodorization of Garlic Sulfur Volatiles

To confirm that pH affects deodorization by altering protein structure, and not by the effects of pH itself, deodorization by water and butter at pH 7 and 4.4 was tested. Both water and fat produced effective deodorization of garlic sulfur volatiles at both pH 4.4 and 7 (Table A11). Fat produced greater deodorization than water because the sulfur volatiles are lipophilic in nature and have stronger interactions with fat than with water [21,22,61]. However, deodorization by water or fat was not affected by pH (Figure 8). Sulfur volatiles are non-ionizable compounds which are generally not affected by pH [21,22,62]. Thus, the pH effect shown in Figure 5 is due to the effect of the pH on the proteins, and not on the volatiles.

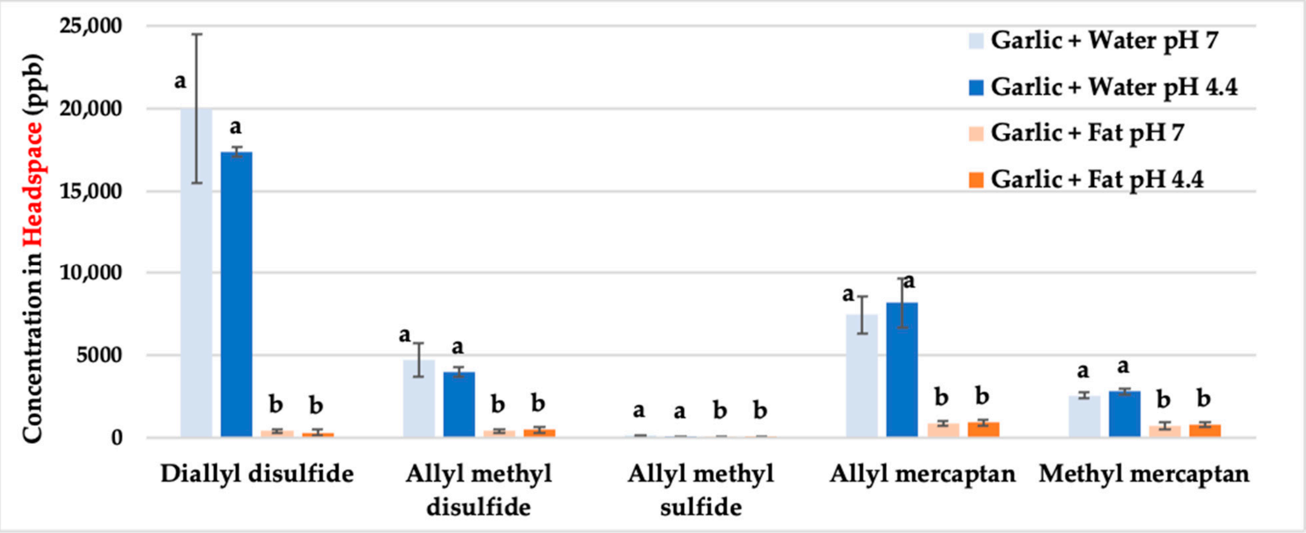

Figure 8. Effects of water and fat pH on deodorization of garlic sulfur volatiles. Treatments within the same volatile with different letters are significantly different ($p < 0.05$).

3.3.3. Effects of Fat, Protein, Fat–Protein Emulsion, and Yogurt on the Deodorization of Garlic Sulfur Volatiles

While fat and protein were effective deodorizers separately, combining them into an emulsion increased their effectiveness in the deodorization of garlic sulfur volatiles (Figure 9; Table A11). When fat is added to milk protein, micellar casein adsorbs on the surface of the fat globules. More binding sites become available when micellar casein is adsorbed on the fat surface because the casein micelle unfolds at the interface [63,64]. Both casein (through alpha S2 and kappa casein) and fat interact with the garlic sulfur volatiles through hydrophobic and disulfide–sulfhydryl interactions [21,22,61] in the emulsion, which results in higher deodorization in the emulsion than in fat or protein alone.

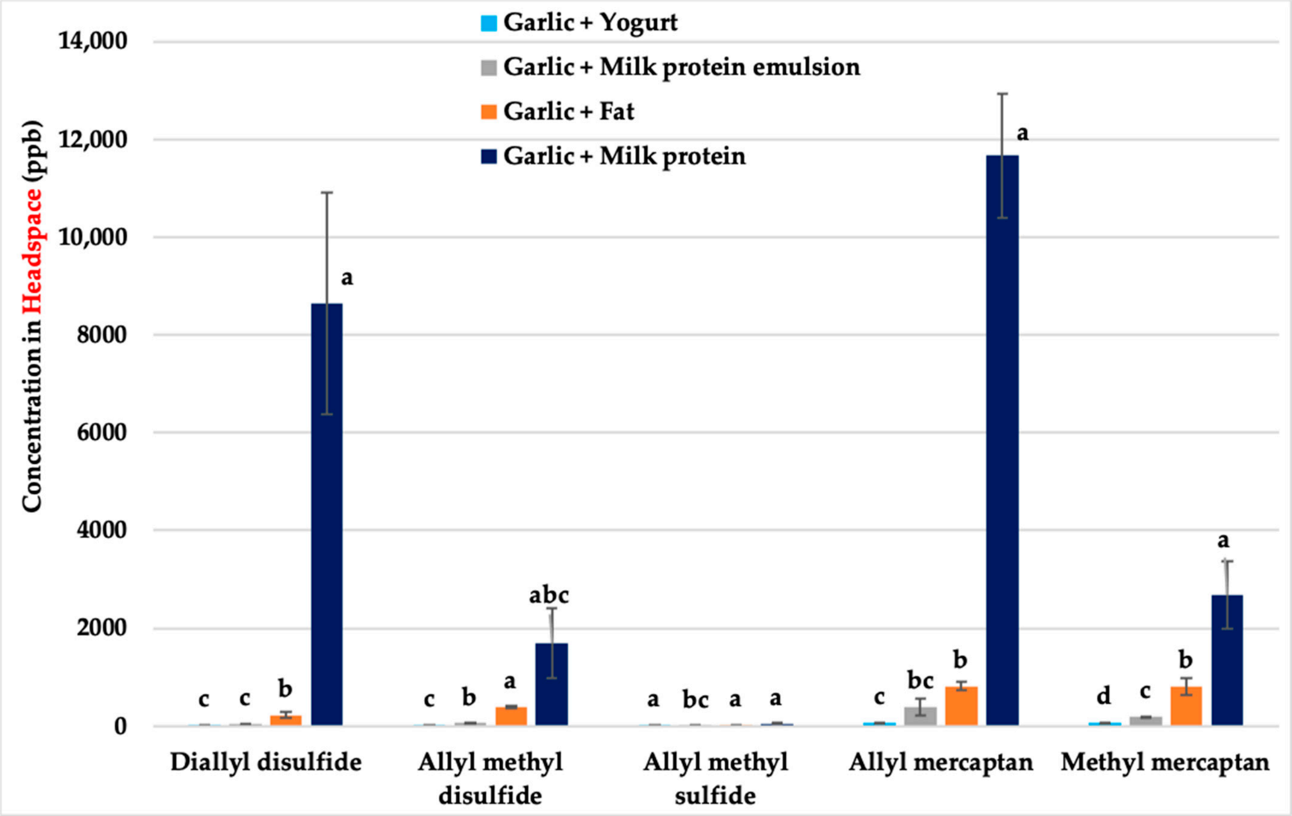

Figure 9. Effects of yogurt, protein, fat, and emulsions at pH 4.4 on deodorization of garlic sulfur volatiles. Treatments within the same volatile with different letters are significantly different ($p < 0.1$).

While an emulsion was more effective in deodorization than were the fat or protein, yogurt had an even greater deodorization ability (Figure 9). This suggests the presence of additional components in yogurt beyond water, fat, proteins, and the emulsion, that contribute significantly to the deodorization of garlic sulfur volatiles.

3.3.4. Effect of Yogurt's Microbial Culture on the Deodorization of Garlic Sulfur Volatiles

To determine if the fermentation process was important to the deodorization of garlic sulfur volatiles, garlic was mixed with non-fat milk and non-fat milk fermented with yogurt culture (*L. acidophilus* and *B. bifidum*). Both treatments showed significant deodorization of garlic sulfur volatiles (Table A12). The non-fat milk containing microbial culture demonstrated a markedly higher deodorization effect compared to the non-fat milk alone, suggesting a crucial role for microbes and their bioproducts in the deodorization process (Figure 10). Both *L. acidophilus* and *B. bifidum* are probiotic bacteria strains [65]. Many studies have shown that probiotic cultures such as *L. acidophilus* and *B. bifidum* can reduce the concentrations of sulfur volatile compounds such as methyl mercaptan [30–32]. There are several possible reasons that the microbial culture may deodorize the volatiles. The metabolites produced during fermentation, including exo-polysaccharides, sphingolipids, and bioactive peptides derived from alpha S1/2-casein, beta-casein, kappa-casein, caseinophosphopeptides, alpha-lactalbumin, beta-lactoglobulin, and lactoferrin [66], may interact with the garlic sulfur volatiles and result in deodorization of these volatiles. Fermentation may expose additional binding sites on the proteins. The increased viscosity of the yogurt due to the exo-polysaccharides and gelation of the proteins [66] may entrap the garlic sulfur volatiles inside the newly formed structure [67] and may also cause deodorization.

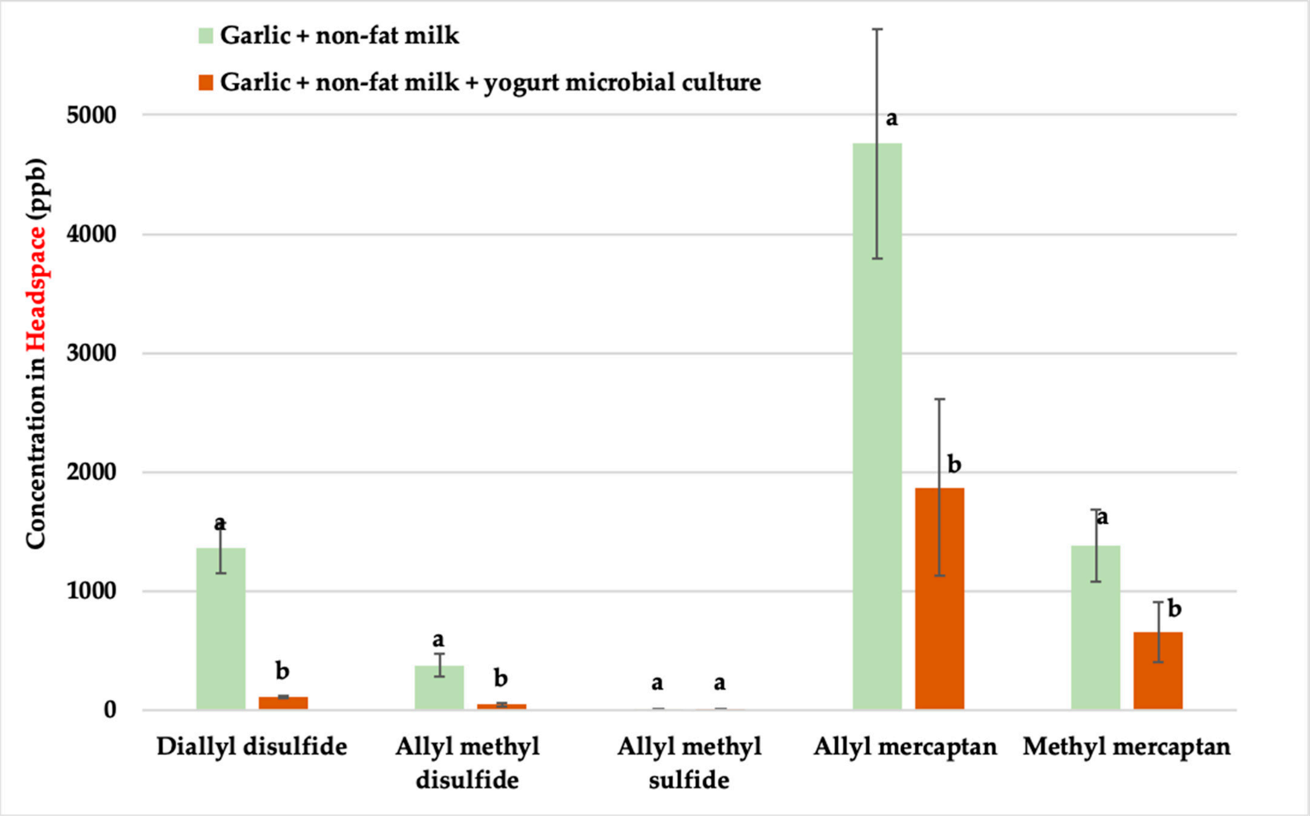

Figure 10. Effects of yogurt microbial culture on deodorization of garlic sulfur volatiles. Treatments within the same volatile with different letters are significantly different ($p < 0.05$).

3.4. Proposed Mechanism for Deodorization of Garlic Sulfur Volatiles by Yogurt

This study proposes that the mechanism for the deodorization of garlic sulfur volatiles is due to the synergistic action of fat, protein, water, and microbial fermentation in the yogurt (Figure 11). Fat binds garlic sulfur volatiles by hydrophobic interactions. Proteins bind the volatiles by disulfide–sulfhydryl interchange reactions and disulfide linkages. In emulsions, protein interacts with the fat, unfolding at the interface and exposing more binding sites. Water partitions the volatiles between garlic, water, and gas phases, reducing their concentration. Fermentation by microbes decreased the pH of yogurt. The acidic pH changes the structure of the proteins, which increases the availability of binding sites for interaction with the garlic sulfur volatiles. The metabolites formed by the microbes during fermentation interact with the garlic sulfur volatiles. Also, the structural changes in the matrix of yogurt and the higher viscosity of the yogurt result in greater interaction of components of yogurt with garlic sulfur volatiles. Overall, the interaction between the components of yogurt and with the garlic sulfur volatiles contributes to the deodorization of sulfur garlic volatiles.

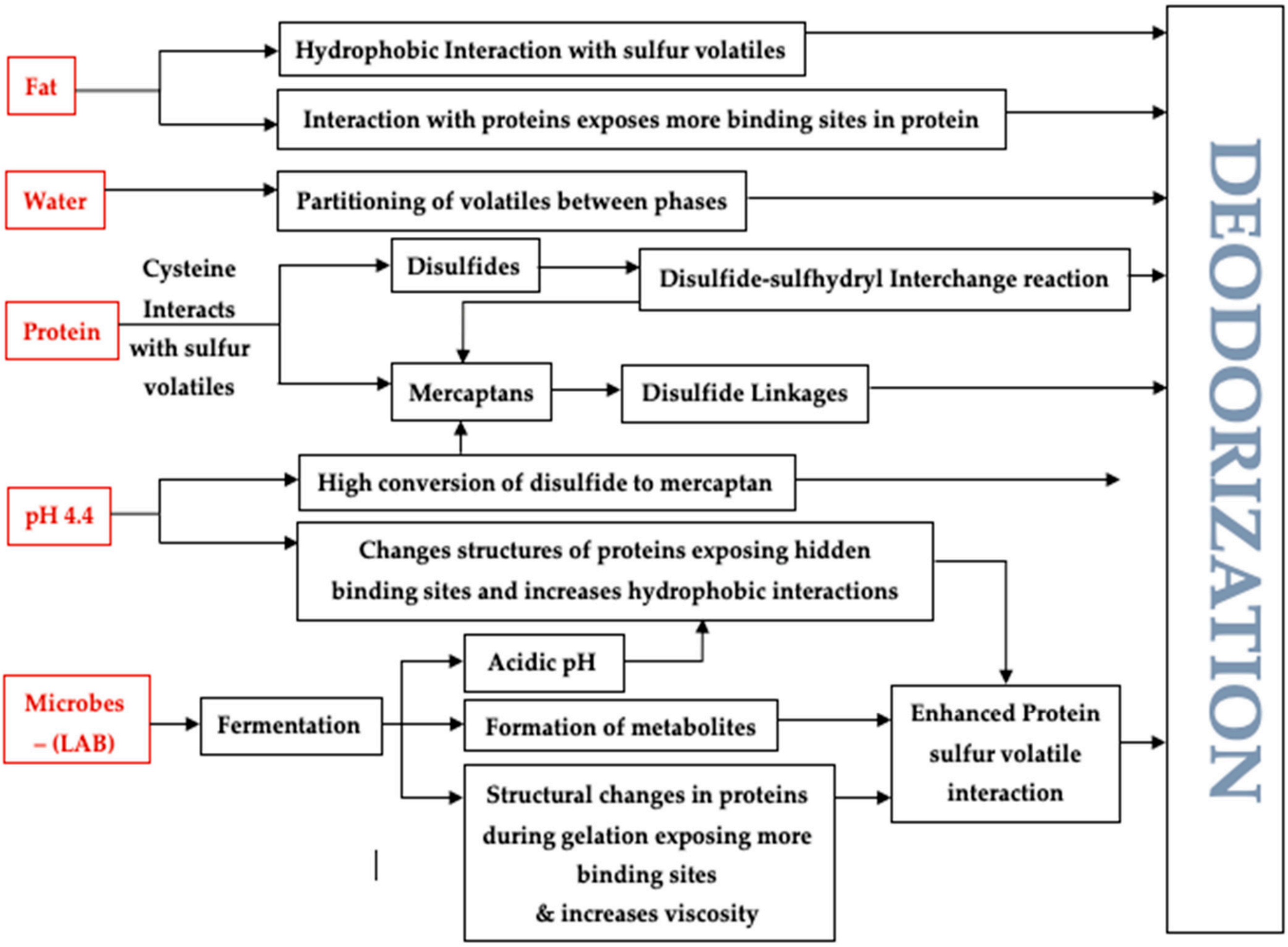

Figure 11. Proposed mechanism of deodorization of garlic sulfur volatiles by yogurt. LAB—Lactic-acid bacteria.

4. Conclusions

Consuming yogurt significantly reduced the concentrations of almost all garlic sulfur volatiles in breath, including diallyl disulfide, allyl methyl disulfide, allyl methyl disulfide, allyl mercaptan, and methyl mercaptan. Consuming yogurt with garlic is the most effective timing for deodorization, followed by consuming yogurt before garlic, and then consuming yogurt after garlic, because interactions before the garlic is swallowed create the greatest deodorization. Yogurt is the most effective deodorizer, followed by its components: protein—fat emulsion, fat, protein, and water. The acidic pH of yogurt changes the structure of the proteins, resulting in a more hydrophobic interaction with the garlic sulfur volatiles. Fermentation by the microbes in the yogurt produces metabolites and creates structural changes in the matrix of the yogurt that may also contribute to deodorization. The findings of this study support the determination of yogurt as a functional food with deodorizing properties, offering a practical solution for individuals who regularly consume garlic. Novel food products with fat, proteins, or prebiotic cultures can be developed targeting garlic sulfur volatiles, with broad application for the mitigation of garlic breath. Future studies will focus on analyzing the effects of different plant-based proteins and LAB on the deodorization of garlic sulfur volatiles.

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Informed Consent Statement: Informed consent was obtained from the subjects involved in the study.

Data Availability Statement: The data presented in the study is available in Appendix A.

Conflicts of Interest: The authors declare no conflicts of interest.

Appendix A

Table A1. Effect of yogurt on diallyl disulfide in breath (ppb).

Time (minutes)	Garlic	Yogurt	Fat	Protein	Water
0	341 ± 122	6.57 ± 1.87	5.87 ± 1.52	8.58 ± 1.52	12.7 ± 9.63
5	113 ± 39.8	4.91 ± 1.17	1.68 ± 0.52	2.65 ± 0.60	7.09 ± 5.15
10	84.2 ± 47.2	2.09 ± 0.53	1.13 ± 0.16	1.69 ± 0.56	2.96 ± 1.74
20	28.7 ± 8.11	0.91 ± 0.06	1.54 ± 0.15	2.09 ± 1.06	2.06 ± 0.79
30	16.0 ± 5.39	0.62 ± 0.17	0.94 ± 0.12	1.53 ± 0.70	1.61 ± 0.34
40	10.0 ± 2.22	0.22 ± 0.08	1.21 ± 0.18	2.12 ± 1.06	1.42 ± 0.46
50	6.77 ± 1.49	0.21 ± 0.15	1.25 ± 0.14	1.22 ± 0.33	1.54 ± 0.39
60	7.48 ± 3.01	0.13 ± 0.08	1.15 ± 0.30	1.09 ± 0.12	1.38 ± 0.31

Table A2. Effect of yogurt on allyl methyl disulfide in breath (ppb).

Time (minutes)	Garlic	Yogurt	Fat	Protein	Water
0	67.0 ± 19.3	0.43 ± 0.12	1.29 ± 0.46	1.14 ± 0.15	2.51 ± 1.73
5	17.0 ± 7.01	0.31 ± 0.11	0.92 ± 0.45	0.77 ± 0.11	1.59 ± 0.33
10	12.0 ± 2.43	0.24 ± 0.26	0.81 ± 0.25	0.76 ± 0.26	1.39 ± 0.14
20	5.47 ± 2.26	0.43 ± 0.60	0.93 ± 0.35	0.82 ± 0.18	1.27 ± 0.17
30	3.77 ± 0.59	0.25 ± 0.02	0.82 ± 0.46	0.90 ± 0.13	1.38 ± 0.30
40	2.94 ± 0.58	0.18 ± 0.08	0.78 ± 0.36	1.12 ± 0.25	1.35 ± 0.32
50	2.80 ± 1.13	0.10 ± 0.04	0.89 ± 0.19	0.85 ± 0.21	1.81 ± 1.32
60	3.00 ± 0.44	0.08 ± 0.02	0.82 ± 0.38	0.82 ± 0.04	1.15 ± 0.11

Table A3. Effect of yogurt on allyl methyl sulfide in breath (ppb).

Time (minutes)	Garlic	Yogurt	Fat	Protein	Water
0	184 ± 51.2	35.4 ± 5.48	18.8 ± 9.92	28.3 ± 8.30	53.4 ± 10.4
5	194 ± 38.8	30.7 ± 6.59	19.5 ± 7.06	35.0 ± 9.14	56.4 ± 12.8
10	206 ± 68.7	23.2 ± 7.43	26.9 ± 7.55	66.1 ± 22.9	74.2 ± 11.3
20	185 ± 88.4	24.2 ± 8.87	28.9 ± 10.3	57.9 ± 13.2	77.7 ± 33.7
30	188 ± 97.5	25.6 ± 7.71	33.1 ± 7.40	54.0 ± 11.9	87.5 ± 41.2
40	192 ± 110	29.3 ± 11.2	34.2 ± 12.3	61.0 ± 15.8	94.2 ± 32.9
50	216 ± 94.8	31.6 ± 12.9	35.7 ± 14.6	57.3 ± 11.5	92.4 ± 19.7
60	207 ± 82.9	33.6 ± 11.8	38.1 ± 14.9	55.6 ± 10.2	97.0 ± 16.2

Table A4. Effect of yogurt on allyl mercaptan in breath (ppb).

Time (minutes)	Garlic	Yogurt	Fat	Protein	Water
0	1567 ± 460	113 ± 26.3	21.0 ± 0.29	41.9 ± 17.7	125 ± 97.7
5	463 ± 145	57.7 ± 9.11	11.0 ± 3.13	11.7 ± 4.00	44.5 ± 35.1
10	333 ± 168	18.5 ± 2.69	14.8 ± 1.27	8.30 ± 2.59	21.9 ± 7.65
20	138 ± 23.4	10.1 ± 5.56	14.7 ± 5.59	7.06 ± 1.42	15.4 ± 3.88
30	85.8 ± 15.8	5.65 ± 2.39	12.5 ± 5.97	6.47 ± 1.58	13.9 ± 3.89
40	68.1 ± 13.1	2.77 ± 1.46	13.7 ± 2.21	7.25 ± 1.79	13.4 ± 4.14
50	53.9 ± 13.3	0.06 ± 1.07	11.0 ± 3.34	6.72 ± 1.94	24.6 ± 22.5
60	43.3 ± 13.2	0.05 ± 0.02	11.2 ± 0.78	5.81 ± 0.61	11.6 ± 4.28

Table A5. Effect of yogurt on methyl mercaptan in breath (ppb).

Time (minutes)	Garlic	Yogurt	Fat	Protein	Water
0	290 ± 26.3	5.52 ± 1.59	3.58 ± 1.18	4.07 ± 0.84	20.0 ± 6.30
5	89.1 ± 28.2	3.20 ± 1.29	2.85 ± 0.68	2.69 ± 0.82	10.6 ± 3.67
10	62.6 ± 10.3	2.05 ± 1.03	4.04 ± 3.39	2.87 ± 1.36	6.98 ± 1.89
20	28.9 ± 8.64	1.45 ± 0.75	2.95 ± 0.94	3.19 ± 0.23	4.90 ± 1.75
30	19.5 ± 3.27	1.35 ± 0.82	2.75 ± 1.44	3.16 ± 0.60	6.22 ± 3.91
40	15.1 ± 2.09	0.80 ± 0.34	4.54 ± 3.31	6.24 ± 4.72	6.00 ± 2.65
50	15.0 ± 4.01	0.48 ± 0.31	3.64 ± 1.03	3.89 ± 1.28	6.37 ± 2.39
60	11.2 ± 3.33	0.14 ± 0.03	3.65 ± 1.54	4.80 ± 3.83	6.65 ± 2.67

Table A6. Effect of time of yogurt consumption on diallyl disulfide in breath (ppb).

Time (minutes)	Garlic	Yogurt before Garlic	Yogurt with Garlic	Yogurt after Garlic
0	341 ± 122	35.2 ± 22.0	6.57 ± 1.87	341 ± 122
5	113 ± 39.8	7.64 ± 2.72	4.91 ± 1.17	10.6 ± 7.95
10	84.2 ± 47.2	3.32 ± 1.18	2.09 ± 0.53	5.07 ± 0.26
20	28.7 ± 8.11	2.07 ± 0.27	0.91 ± 0.06	2.92 ± 0.15
30	16.0 ± 5.39	1.56 ± 0.14	0.62 ± 0.17	1.88 ± 0.22
40	10.0 ± 2.22	1.44 ± 0.08	0.22 ± 0.08	1.23 ± 0.03
50	6.77 ± 1.49	1.29 ± 0.06	0.21 ± 0.15	1.01 ± 0.17
60	7.48 ± 3.01	1.17 ± 0.07	0.13 ± 0.08	0.96 ± 0.11

Table A7. Effect of time of yogurt consumption on allyl methyl disulfide in breath (ppb).

Time (minutes)	Garlic	Yogurt before Garlic	Yogurt with Garlic	Yogurt after Garlic
0	67.0 ± 19.3	5.30 ± 3.69	0.43 ± 0.12	67.0 ± 19.3
5	17.0 ± 7.01	1.57 ± 0.33	0.31 ± 0.11	4.11 ± 1.82
10	12.0 ± 2.43	1.28 ± 0.12	0.24 ± 0.26	1.22 ± 0.62
20	5.47 ± 2.26	1.14 ± 0.09	0.43 ± 0.60	0.94 ± 0.22
30	3.77 ± 0.59	0.98 ± 0.07	0.25 ± 0.02	1.00 ± 0.19
40	2.94 ± 0.58	1.03 ± 0.10	0.18 ± 0.08	0.95 ± 0.14
50	2.80 ± 1.13	0.91 ± 0.09	0.10 ± 0.04	1.00 ± 0.21
60	3.00 ± 0.44	0.84 ± 0.02	0.08 ± 0.02	0.95 ± 0.08

Table A8. Effect of time of yogurt consumption on allyl methyl sulfide in breath (ppb).

Time (minutes)	Garlic	Yogurt before Garlic	Yogurt with Garlic	Yogurt after Garlic
0	184 ± 51.2	49.7 ± 5.48	35.4 ± 5.48	184 ± 51.2
5	194 ± 38.8	31.3 ± 1.94	30.7 ± 6.59	66.8 ± 13.7
10	206 ± 68.7	35.3 ± 2.53	23.2 ± 7.43	71.2 ± 21.1
20	185 ± 88.4	35.9 ± 2.58	24.2 ± 8.87	79.6 ± 25.5
30	188 ± 97.5	37.5 ± 0.81	25.6 ± 7.71	78.7 ± 25.4
40	192 ± 110	41.7 ± 0.49	29.3 ± 11.2	70.9 ± 28.1
50	216 ± 94.8	44.3 ± 2.85	31.6 ± 12.9	72.5 ± 23.6
60	207 ± 82.9	45.5 ± 0.38	33.6 ± 11.8	75.7 ± 19.6

Table A9. Effect of time of yogurt consumption on allyl mercaptan in breath (ppb).

Time (minutes)	Garlic	Yogurt before Garlic	Yogurt with Garlic	Yogurt after Garlic
0	1567 ± 460	195 ± 130	113 ± 26.3	1567 ± 460
5	463 ± 145	35.1 ± 12.4	57.7 ± 9.11	134 ± 21.3
10	333 ± 168	15.1 ± 2.71	18.5 ± 2.69	31.1 ± 0.53
20	138 ± 23.4	9.22 ± 1.65	10.1 ± 5.56	17.5 ± 4.08
30	85.8 ± 15.8	7.29 ± 0.87	5.65 ± 2.39	12.4 ± 1.81
40	68.1 ± 13.1	7.19 ± 1.13	2.77 ± 1.46	8.38 ± 0.49
50	53.9 ± 13.3	4.90 ± 0.49	0.06 ± 1.07	7.77 ± 0.49
60	43.3 ± 13.2	4.41 ± 0.75	0.05 ± 0.02	7.20 ± 0.43

Table A10. Effect of time of yogurt consumption on methyl mercaptan in breath (ppb).

Time (minutes)	Garlic	Yogurt before Garlic	Yogurt with Garlic	Yogurt after Garlic
0	290 ± 26.3	30.4 ± 15.6	5.52 ± 1.59	290 ± 26.3
5	89.1 ± 28.2	9.39 ± 2.23	3.20 ± 1.29	13.9 ± 1.29
10	62.6 ± 10.3	4.65 ± 1.34	2.05 ± 1.03	4.37 ± 0.86
20	28.9 ± 8.64	3.18 ± 0.06	1.45 ± 0.75	4.98 ± 0.95
30	19.5 ± 3.27	3.04 ± 0.40	1.35 ± 0.82	4.21 ± 0.90
40	15.1 ± 2.09	2.39 ± 0.34	0.80 ± 0.34	3.37 ± 1.01
50	15.0 ± 4.01	2.23 ± 0.08	0.48 ± 0.31	2.76 ± 0.69
60	11.2 ± 3.33	2.31 ± 0.15	0.14 ± 0.03	2.36 ± 0.63

Table A11. Effects of fat, protein, and water and pH on the deodorization of garlic sulfur volatiles (ppb).

Volatiles	Diallyl Disulfide	Allyl Methyl Disulfide	Allyl Methyl Sulfide	Allyl Mercaptan	Methyl Mercaptan
Garlic	347,015 ± 177,444	66,016 ± 34,776	13,369 ± 7068	418,377 ± 124,214	40,383 ± 7543
Yogurt pH 4.4	12.0 ± 2.40	14.8 ± 5.02	21.7 ± 1.68	60.3 ± 8.29	66.2 ± 9.82
Water pH 7	20,027 ± 4510	4714 ± 990	88.3 ± 11.8	7451 ± 1123	2531 ± 188
Water pH 4.4	17,359 ± 281	3971 ± 287	81.7 ± 3.94	8187 ± 1504	2786 ± 181
Fat pH 7	419 ± 71.6	426 ± 82.9	29.8 ± 8.4	380 ± 171	379 ± 90.1
Fat pH 4.4	221 ± 59.2	385 ± 27.3	26.7 ± 3.13	814 ± 84.2	803 ± 164
WPC pH 7	2458 ± 440	1118 ± 69.6	25.5 ± 4.24	1291 ± 437	1472 ± 369
WPC pH 4.4	1925 ± 202	835 ± 35.5	29.1 ± 1.24	4944 ± 1229	1755 ± 109
WPI pH 7	6554 ± 1039	1758 ± 205	36.0 ± 2.24	2520 ± 321	961 ± 85.5
WPI pH 4.4	4066 ± 1440	678 ± 110	39.1 ± 6.27	11,208 ± 2106	1999 ± 213

Table A11. *Cont.*

Volatiles	Diallyl Disulfide	Allyl Methyl Disulfide	Allyl Methyl Sulfide	Allyl Mercaptan	Methyl Mercaptan
CC pH 7	8921 ± 1843	2888 ± 364	56.6 ± 7.83	4177 ± 490	1665 ± 144
CC pH 4.4	4303 ± 1171	935 ± 335	39.7 ± 10.5	7673 ± 815	1903 ± 287
MC pH 7	14,344 ± 2405	6948 ± 1241	141 ± 28.5	2089 ± 162	1625 ± 133
MC pH 4.4	6573 ± 1828	1101 ± 360	44.9 ± 11.6	12,041 ± 1831	2606 ± 733
MPC pH 7	10,821 ± 1634	5084 ± 706	101 ± 19.6	1575 ± 95.8	1168 ± 158
MPC pH 4.4	8645 ± 2262	1693 ± 718	52.9 ± 12.7	11,670 ± 1269	2670 ± 688
MPI pH 7	14,254 ± 1290	6461 ± 427	106 ± 7.26	1723 ± 251	1510 ± 428
MPI pH 4.4	11,454 ± 2115	3210 ± 366	92.5 ± 13.1	14,728 ± 1674	3896 ± 429
WPC-Fat emulsion pH 4.4	110 ± 8.11	196 ± 60.1	33.7 ± 4.75	8062 ± 1156	2531 ± 201
MPC-Fat emulsion pH 4.4	39.5 ± 12.5	55.9 ± 11.8	10.2 ± 1.43	390 ± 180	194 ± 3.78

Table A12. Effect of yogurt microbial culture on deodorization of sulfur volatiles in raw garlic (ppb).

Volatiles	Garlic	Non-Fat Milk	Microbial Culture in Non-Fat Milk
Diallyl disulfide	85,150 ± 17,591	1364 ± 213	110 ± 8.96
Allyl methyl disulfide	24,920 ± 5154	379 ± 94.1	49.8 ± 18.2
Allyl methyl sulfide	1578 ± 376	14.6 ± 2.17	14.5 ± 0.99
Allyl mercaptan	146,965 ± 7961	4758 ± 965	1872 ± 741
Methyl mercaptan	33,849 ± 1971	1382 ± 299	658 ± 256

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