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DEODORIZATION OF GARLIC ODOR BY FRESH AND DRIED HERBS USING SIFT-MS

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Ozcan-Sinir, G., Barringer, S.A. (2021). Sarımsak kokusunun taze ve kuru bitkiler ile SIFT-MS kullanılarak deodorizasyonu. GIDA (2021) 46(2) 358-366 doi: 10.15237/gida.GD21029

ABSTRACT
Garlic (*Allium sativum* L.) has a very strong odor, which lingers on the breath for approximately a day, after consumption. The deodorization mechanism for garlic odor has been associated with the phenolic content and enzymatic activity of the deodorizing food. In this study, the effectiveness of both fresh and dried form of the herbs (mint, oregano, rosemary and thyme) were examined to reduce the garlic volatile content of allyl mercaptan, allyl methyl disulfide, allyl methyl sulfide and diallyl disulfide. The concentration of garlic volatiles was measured in the headspace for 30 min by Selected Ion Flow Tube Mass Spectrometry (SIFT-MS). In general, dried herbs were found to be more effective at deodorizing selected volatiles than fresh herbs. Fresh rosemary had the strongest deodorization effect among the fresh herbs, while dried mint had the strongest effect among the dried herbs. Fresh thyme had the lowest effectiveness on deodorization of garlic volatiles.
Keywords: *Allium sativum* L., volatile sulfur compounds, deodorization, SIFT-MS, rosemary, mint

SARIMSAK KOKUSUNUN TAZE VE KURU BİTKİLER İLE SIFT-MS KULLANILARAK DEODORİZASYONU

ÖZ
Sarımsağın (*Allium sativum* L.) tüketimden yaklaşık bir gün sonra nefeste kalan çok güçlü bir kokusu vardır. Sarımsak kokusunun giderilme mekanizması, koku giderici gıdanın fenolik içeriği ve enzimatik aktivitesi ile ilişkilendirilmiştir. Bu çalışmada, çeşitli bitkilerin (nane, kekik, biberiye ve kekik) hem taze hem de kuru formunun çiğ sarımsaktaki uçucu bileşiklerden alil merkaptan, alil metil disülfür, allil metil sülfür ve dialil disülfidin içeriğinin azaltılması üzerine etkinliği incelenmiştir. Sarımsaktaki uçucu bileşenlerin konsantrasyonu, seçilen iyon akış tüpü kütle spektrometresi (SIFT-MS) ile 30 dakika boyunca tepe boşluğundan ölçülmüştür. Genel olarak, kurutulmuş bitkilerin, seçili uçucu bileşenlerden kaynaklanan kokularının giderilmesinde taze bitkilerden daha etkili olduğu saptanmıştır. Taze biberiye, taze otlar arasında en güçlü koku giderme etkisine sahipken, kurutulmuş otlar arasında en güçlü etkiyi kurutulmuş nane göstermiştir. Taze kekik, sarımsak uçucularının deodorizasyonunda en düşük etkiyi göstermiştir.
Anahtar kelimeler: *Allium sativum* L., uçucu sulfur bileşenleri, deodorizasyon, SIFT-MS, biberiye, nane

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INTRODUCTION

Garlic (*Allium sativum* L., Family Liliaceae) is one of the most valuable vegetables in the world. The overall harvested fields of dry bulbs are 1.634.634 ha and the yearly production is 30.708.243 tonnes (FAO, 2019). Garlic has been commonly consumed as food and medicine all over the world since ancient times. It produces enhancements in culinary, therapeutic, and medicinal areas. Some research shows that garlic lowers blood cholesterol and blood sugar (Thomson vd., 2006), and acts as an antiaging agent, which improves memory (Kim vd., 2013). It is a good source of several vitamins and minerals, also it helps in lowering blood pressure and cholesterol and reduces the risk of cancer (Block vd., 1992; Yeh ve Liu, 2001).

Garlic is consumed as a raw or dried vegetable, garlic oil, garlic extract and garlic powder. Regardless of its beneficial effects, garlic consumption ends up producing a strong and lingering odor, which may stay up to 24 h on the breath after consumption (Mirondo ve Barringer, 2016). High levels of volatile sulfur compounds are responsible for the distinctive odor and flavor (Lawson, 1996). The formation of the characteristic flavor of garlic is a consequence of several biochemical reactions, which produce volatile organosulfur compounds and non-volatile amino acids (Amagase vd., 2001; Martins vd., 2016). The major volatile compounds responsible for garlic breath are diallyl disulfide, allyl mercaptan, allyl methyl disulfide, and allyl methyl sulfide (Suarez vd., 1999; Rosen vd., 2001; Tamaki vd., 2008). Even though the precursors of these volatile compounds are present inside the cells, formation doesn't start until garlic is crushed or chopped, when the conversion of alliin to allylsulfenic acid to allicin occurs by the enzyme alliinase. Allicin converts to allyl methyl disulfide and diallyl disulfide (Negishi vd., 2002). Allyl methyl disulfide originates from allicin and does not convert into other garlic volatiles (Suarez vd., 1999). Diallyl disulfide is reduced to form allyl mercaptan (Iciek vd., 2009). These volatiles are formed in raw garlic; only allyl methyl sulfide can be formed both in garlic and inside of the body (Lawson, 1998; Hansanugrum ve Barringer, 2010).

Researchers have proposed that the deodorization of garlic odor is connected to the total amount of phenolic constituents in several fruits and vegetables, and that polyphenol oxidase (PPO) and peroxidase (POD) are the main enzymes present in plant tissues responsible for the reactions (Negishi ve Ozawa, 1997; Negishi ve Negishi, 1999; Negishi vd., 2002). The suggested deodorization process involves the reaction of phenolic radicals with garlic volatiles. Oxidation of the phenolic compounds results in the development of radical quinones that bind with thiols and other organosulfur compounds. This process leads to formation of odorless phenol-organosulfur complexes. The enzymes PPO or POD increase the rate of oxidation of phenolic compounds, and further increase the ensuing addition reaction of volatile compounds to radical quinones (Yasuda ve Onogi, 1996; Negishi ve Negishi, 1999; Negishi vd., 2002). For that reason, a higher deodorization effect will be observed in the presence of both enzymes and phenolic compounds. This mechanism is not completely understood, but current studies have focused on the effectiveness of food and food components, including tea and tea-like beverages, raw fruits, raw vegetables, and some dairy products on lowering the level of the volatile compounds related to malodorous odor (Yasuda ve Arakawa, 1995; Negishi vd., 2002; Negishi vd., 2004; Hansanugrum ve Barringer, 2010; Munch ve Barringer, 2014; Mirondo ve Barringer, 2016; Castada vd., 2017).

The aim of present study was to compare the deodorization effectiveness of mint, oregano, rosemary, and thyme, both dried and fresh, by measuring the reduction in headspace concentration of allyl mercaptan, allyl methyl disulfide, allyl methyl sulfide, and diallyl disulfide with SIFT-MS.

MATERIALS AND METHODS

Materials

Garlic, fresh thyme, oregano, mint, and rosemary, and dried thyme were obtained from a local market (Giant Eagle, Columbus, Ohio, U.S.A.). Dried oregano, mint, and rosemary were obtained from a spice bazaar (Bursa, Turkey). All materials were stored at room temperature until use.

Headspace volatile measurements

5 g peeled garlic cloves (raw), 5 g fresh herb or the equivalent amount of dried herb to produce the same dry weight content as the fresh sample, and 50 mL of HPLC grade water were blended (Magic Bullet model MB1001B, Ningbo Great Height Commodity, Ningbo, China) for 30 sec. The same procedure was also applied to control measurements without herb addition. The blended mixture was transferred in 500 mL Schott glass bottles and covered with a Teflon sealed screw cap. After capping, the headspace volatiles of the mixture were analyzed using selected ion flow tube mass spectrometry (SIFT-MS) immediately. Volatile compound measurements were conducted at room temperature (25±1°C) from 0 to 30 min at 5 min intervals. Total SIM scan time was 120 s.

Selected ion flow tube mass spectrometry (SIFT-MS)

Table 1 outlines the volatile compounds analyzed in this study with their corresponding ion product, precursor ions, mass-to-charge ratios (*m/z*), and the reaction rates used in SIFT-MS. SIFT-MS utilizes chemical ionization with H_3O^+, NO^+ and O_2^+. The concentration (M) of volatile compounds were obtained via product count rate (Ip), reaction rate constant (k), precursor ions count rate (I), and reaction time (t) as follows: (M) = Ip/Ikt (Spaněl ve Smith, 1999). Trace volatile analyte compounds were introduced in the reactor at an optimized sample inlet flow rate of 0.35 Torr•L/s (26 cm^3/min). The validation was performed before usage of the instrument by quantifying properly via linearity, range, accuracy, precision and limit of detection using a pressurized mixture of certified gas standards (benzene, ethylene, isobutane, octafluorotoluene, hexafluorobenzene, toluene, p-xylene, and 1,2,3,4-tetrafluorobenzene) each having a concentration of 2 ppm (±5%) in nitrogen (Air Liquide America Specialty Gases LLC, Plumsteadville, PA, USA) and standardized to a pressure of 21 kPa. Conflicts between selected volatiles were removed by selecting different masses or different precursor ions for each compound in the method.

Table 1. Detailed SIFT-MS information of measured volatile compounds

Volatile Compound	Ion Product	Precursor Ion	*m/z*	Reaction rate (*k*) (10^{-9} cm^3/s)
Allyl mercaptan	C_3H_6S	NO^+	74	2.4
Allyl methyl disulfide	$C_4H_8S_2$	NO^+	120	2.4
Allyl methyl sulfide	$C_4H_8S.H^+$	H_3O^+	89	2.6
Diallyl disufide	$(C_3H_5)_2S_2^+$	NO^+	146	2.4

Statistical analysis

The Shapiro-Wilk test was applied to assess the normality of the distribution of variables. The Kruskal-Wallis test was employed to determine whether significant differences existed among the groups. The Mann-Whitney U-test was used for comparing two groups after the Kruskal Wallis test was performed, in cases in which significances was determined. The Mann-Whitney U-test was applied for comparing the continuous variables not meeting normality assumption. Continuous variables were presented as the median. The significance level was set to $\alpha = 0.05$. Statistical analyses were performed with IBM SPSS Statistics version 25.0 (IBM Corporation, New York, USA).

RESULTS AND DISCUSSION

Mint, oregano, rosemary and thyme showed a very significant deodorization capacity against allyl mercaptan, allyl methyl disulfide, allyl methyl sulfide and diallyl disulfide (Figures 1-4). These volatile compounds produce the aroma unique to garlic and have been measured in breath and headspace analyses by several researchers (Hansanugrum ve Barringer 2010; Munch ve Barringer, 2014; Mirondo ve Barringer, 2016). Control samples, without addition of an herb, had a continuous increase in the concentration of measured garlic volatiles over time, because of the continued enzymatic formation of volatile sulfur compounds (Figures 1-4). When garlic is cut, ground or crushed, the enzyme allinase is released and hydrolyzes alliin, a non-proteinogenic amino acid. Several enzymatic reactions follow, resulting in formation of allicin, which has a garlic odor, and is the main precursor for the other volatiles responsible for garlic odor. Allicin rapidly degrades into other sulfur compounds (Lawson, 1996; Negishi vd., 2002).

In general, dried herbs were at least twice as effective as fresh herbs, at deodorizing the volatile compounds in garlic. This may be explained by the higher total phenolic content in dry herbs than fresh herbs, at the same total dry matter content. Vaidya vd. (2014) investigated the total phenolic content and antioxidant activity of fresh and dried rosemary. They determined that dried samples had higher total phenolic content and antioxidant activity compared to fresh samples. Sharma vd. (2020) investigated several extraction method to identify differences in bioactive compound content in rosemary. Caffeic acid, rosmarinic acid, luteolon-7-o-glucoside, carnosic acid, carnosol, and ursolic acid content was significantly higher in the dry leaf decoction compared to the fresh leaf decoction. Hossain vd. (2010) investigated total phenolic content, rosmarinic acid, and antioxidant capacity of dried rosemary, oregano, marjoram, sage, basil, and thyme after drying and compared to fresh samples. They determined that the fresh samples had lower total phenolic content, rosmarinic acid, and antioxidant capacity than the dry samples.

The higher levels of phenolics and terpenoids in dry herbs compared to fresh herbs, may be explained by the difference in biomass that can be extracted. Dried samples have a higher extracted compound concentration, due to the fragility of the tissues. The drying process makes tissues more fragile, so cell walls break more easily during the extraction procedure. These broken cells result in higher extraction of the phenolic compounds by the solvents during the process (Hossain vd., 2010).

Enzymatic degradation is another possible explaination for the lower antioxidant capacity of fresh herbs. Fresh samples have active enzymes which can cause degradation during processing. To maximize antioxidant extraction, it is recommended to use dried or frozen herbs, rather than fresh samples, due to enzymatic activity (Suhaj, 2006). Many enzymes are inactivated by decreased water activity, thus dried samples maintain a higher antioxidant capacity and total phenolic content in herbal extracts.

Fresh rosemary had the highest deodorization effect among the fresh herbs, while all of the dried herbs were equally effective (Figure 1-4). The main phenolic compound in rosemary is rosmarinic acid, which has a higher trolox equivalent antioxidant capacity than other phenolic compounds such as catechin and quercetin (Berker vd., 2013). However, fresh thyme had the lowest effectiveness in deodorization of garlic volatiles (Table 2). The total lipid content of fresh rosemary is more than twice that of fresh thyme (USDA, 2021). Oil significantly lowers the volatility of garlic volatiles in the headspace due to the hydrophobicity of the volatiles (Hansanugrum ve Barringer, 2010). Thus the oil content may contribute to the better deodorization effect of fresh rosemary. Mirondo ve Barringer (2016), reported the deodorizing effect of fresh spearmint leaves against garlic breath volatiles. Munch ve Barringer (2014) also reported that parsley, spinach, and mint leaf applications were all efficient in removing allyl methyl disulfide, diallyl disulfide, allyl mercaptan, and allyl methyl sulfide, which are malodorous garlic breath volatiles by enzymatic deodorization. The dried mint was significantly effective at deodorizing all measured volatiles (Table 2).

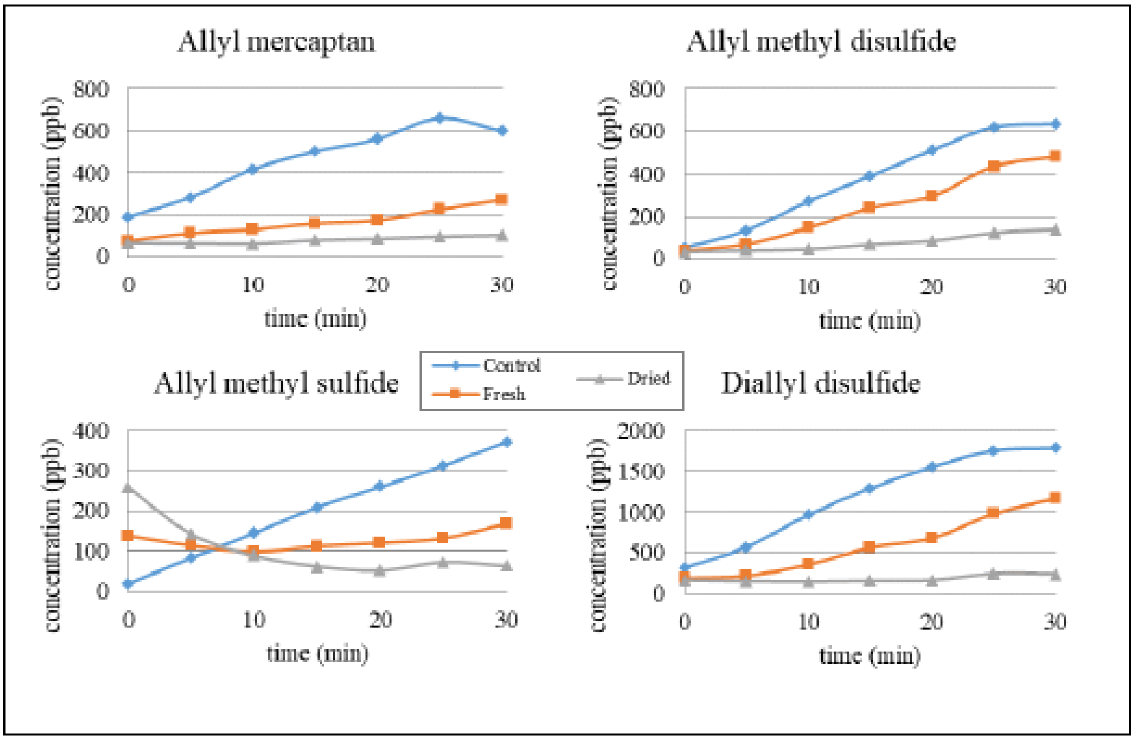

Figure 1. Effect of fresh and dried mint on concentration changes of selected volatiles

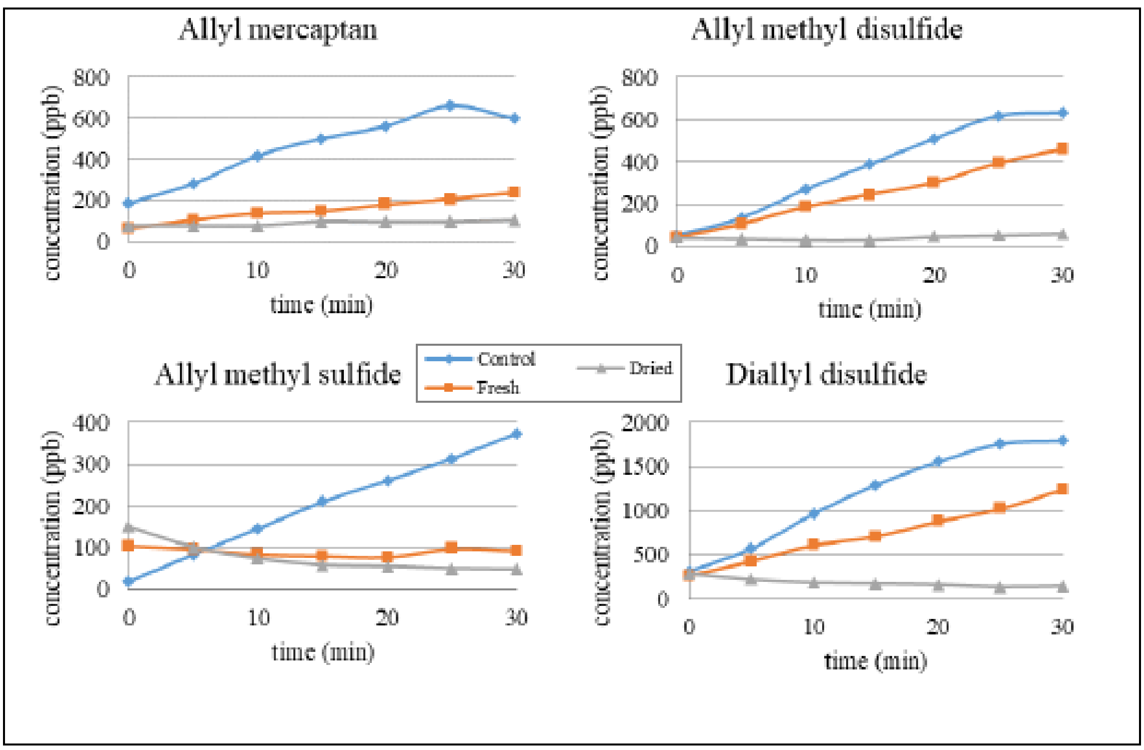

Figure 2. Effect of fresh and dried oregano on concentration changes of selected volatiles

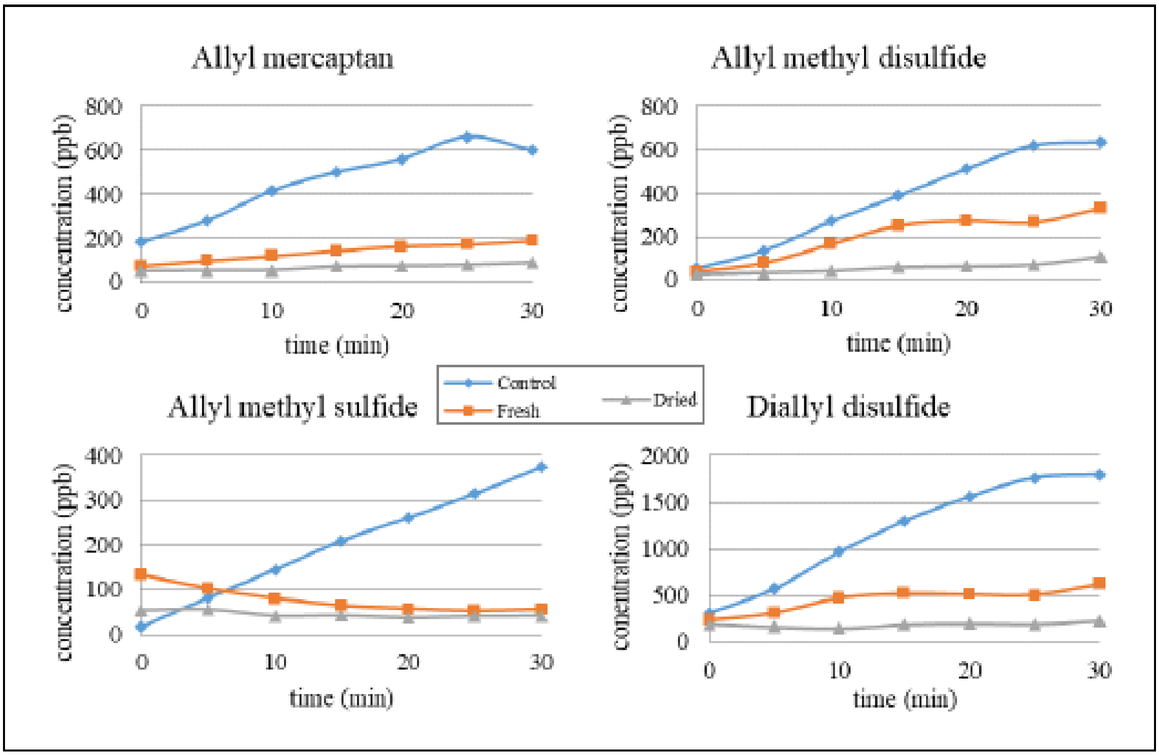

Figure 3. Effect of fresh and dried rosemary on concentration changes of selected volatiles

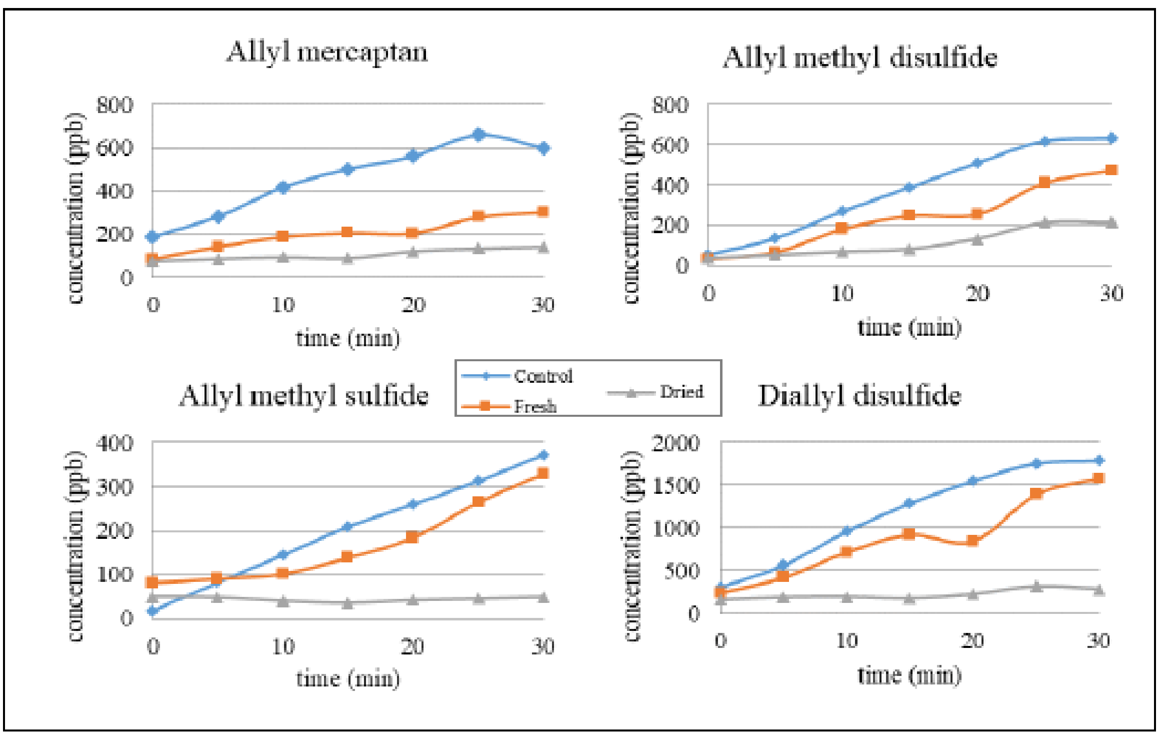

Figure 4. Effect of fresh and dried thyme on concentration changes of selected volatiles

Table 2. *P*-value of difference in garlic volatile concentration at 30 min, for fresh vs. dried herb, fresh herb vs. control, and dried herb vs. control

		Allyl mercaptan	Allyl methyl disulfide	Allyl methyl sulfide	Diallyl disulfide
Fresh vs. Dried Herb	Mint	0.014	0.025	0.014	0.014
	Oregano	0.007	0.011	>0.05	0.011
	Rosemary	0.013	0.013	>0.05	0.013
	Thyme	0.025	>0.05	0.025	0.025
Fresh Herb vs. Control	Mint	0.007	>0.05	>0.05	0.050
	Oregano	0.005	>0.05	0.024	0.050
	Rosemary	0.005	>0.05	0.031	0.050
	Thyme	0.050	>0.05	>0.05	>0.05
Dried Herb vs. Control	Mint	0.034	0.034	0.034	0.034
	Oregano	0.034	0.034	0.034	0.034
	Rosemary	0.034	0.034	0.034	0.034
	Thyme	0.034	0.034	0.034	0.034

CONCLUSION

Mint, oregano, rosemary and thyme significantly reduced the headspace volatile sulfur compounds present in garlic and suppressed malodorous odor. Fresh rosemary had the highest deodorization effect among the fresh herbs, while dried mint had the strongest effect among the dried herbs. Fresh thyme had the lowest effectiveness on deodorization of garlic volatiles. In general, the dried forms of these herbs were found to be more effective than the fresh herbs. This may be due to a drying process effect, which causes cell walls to break more easily and expose more phenolic compounds. Another reason may be fractionation of phenolic compounds into smaller components which have higher antioxidant activity, or higher enzymatic degradation of phenolic compounds in fresh herbs. In conclusion, the present study suggests that the odor of garlic volatiles can be reduced by herbs, especially dried herbs.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Gülşah OZCAN-SINIR: methodology, investigation, data curation, writing-original draft. Sheryl Ann BARRINGER: supervision, resources, investigation, writing - review & editing. All authors approved the submitted version.

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