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EFFECTS OF BLACK PEPPER AND SODIUM ASCORBATE ON VOLATILE COMPOUNDS OF SUCUK

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ABSTRACT

The effects of different levels of black pepper (5, 10 or 15 g/kg) and the use of ascorbate (without ascorbate and with ascorbate) on the volatile compounds of sucuk were examined in the study. The volatile compounds of the samples belonging to each group were extracted by solid phase micro-extraction (SPME) and analyzed by gas chromatography-mass spectrometry (GC-MS). Ascorbate was not effective on aldehydes, aromatic hydrocarbons, esters, acids and terpenes. While high black pepper level (15 g/kg) decreased the abundance of hexanal, it increased the ethanol abundance. α -pinene and 3-carene increased as black pepper levels increased. Among the terpenes, D-limonene and caryophyllene were the most abundant compounds and were significantly affected by the black pepper level of 15 g/kg. D-limonene and caryophyllene were the most related compounds for PC1; while allyl mercaptane, γ-terpinene, D-limonene, hexane for PC2.

Keywords: Volatile compound, black pepper, ascorbate, fermented sausage, sucuk, principal component analysis (PCA)

KARABİBER VE SODYUM ASKORBATIN SUCUĞUN UÇUCU BİLEŞİKLERİ ÜZERİNE ETKİLERİ

ÖZ

Araştırmada, farklı seviyelerde karabiber (5, 10 veya 15 g/kg) ve askorbat (askorbatsız ve askorbatlı) kullanımının sucuğun uçucu bileşikleri üzerindeki etkileri incelenmiştir. Her gruba ait numunelerin uçucu bileşikleri katı faz mikro ekstraksiyon (SPME) ile ekstrakte edilmiş ve gaz kromatografi-kütle spektrometrisi (GC-MS) ile analiz edilmiştir. Askorbat, aldehitler, aromatik hidrokarbonlar, esterler, asitler ve terpenler üzerinde etkili olmamıştır. Yüksek karabiber düzeyi (15 g/kg) hekzanal seviyesini azaltırken, etanol seviyesini artırmıştır. Karabiber seviyesi arttıkça α-pinen ve 3-caren artmıştır. Terpenler içerisinde D-limonen ve karyofillen en fazla bulunan bileşikler olup 15 g/kg karabiber seviyesinden önemli ölçüde etkilenmiştir. D-limonen ve karyofillen PC1, allil merkaptan, γ-terpinen, D-limonen ve hekzan ise PC2 için en ilgili bileşiklerdir.

Anahtar Kelimeler: Uçucu bileşik, karabiber, askorbat, fermente sosis, sucuk, temel bileşen analizi (PCA)

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INTRODUCTION

Flavor is one of the most important quality attributes for meat products and contributes the acceptability of the product by consumer. The biochemical changes during ripening of fermented sausages determine the taste and aroma of the final product. Carbohydrate fermentation, lipolysis, proteolysis, lipid oxidation and amino acid catabolism are important phenomena with regard to the characteristic flavor of dry and semi-dry fermented sausages such as sucuk, salami, Rohwurst, Chorizo, pepperoni (Ordonez et al., 1999; Kaban, 2010; Kaya and Kaban, 2019). In these reactions, external factors such as the initial fermentation temperature, as well as internal factors such as salt, curing agent, sugar and spices, which are involved in the formulation, have become effective. The abundance and balance of volatile and non-volatile compounds formed as a result of the reactions vary depending on the type of product, and thus different products can be obtained in terms of flavor (Kaya and Kaban, 2019). Volatile compounds contribute to the aroma of the product, while non-volatile compounds such as peptides and free amino acids directly affect the flavor of the product (Toldra, 1998).

The spices used in fermented sausages also contribute to the characteristic flavor and aroma of the product. In the formulation, spices are used in proportions varying between 0.5-2% depending on the type of product, and even some products are characterized by the specific spices they contain (Ordonez et al., 1999). Black pepper takes place an important position among the spices and it is called "the king of the spices" (Srınıvasan, 2007; Zhang et al., 2021). Black pepper is widely used in fresh meat and processed meat products because of its pungent and characteristic taste (Vignolo et al., 2010). Black pepper is also an important spice for sucuk, a type of dry fermented sausage. However, there is no study of the effect of black pepper on sucuk's volatile profile.

The additives also play an important role on flavor of fermented sausages. Salt and nitrite are essential additives for these products. Besides, ascorbate can be also added to the formulation. However, the number of studies on the effect of this reducing compound on the volatile compound profile of fermented sausages is rather limited (Stahnke, 1999). The relationship between the volatile compound profile and ascorbate in sucuk has not yet been investigated (Kaban and Kaya, 2009; Ekici et al., 2015; Yalınkılıç et al., 2015). Therefore, it is aimed to determine the effects of different levels of black pepper (5 g/kg, 10 g/kg or 15 g/kg) and the use of ascorbate (with or without sodium ascorbate) on the volatile compound profile of sucuk, a type of dry fermented sausage.

MATERIAL AND METHOD

Material

Beef meat (round) and beef fat were used as a raw material obtained from two carcasses (24 h post-mortem). After raw meat was trimmed of visible fat and connective tissue, it was cut into small pieces. Similarly, beef fat was also cut into small pieces. Then, after vacuum packaging, they were separately stored at -18 °C for a week.

Autochthonous strains (*Lactobacillus plantarum* GM77 + *Staphylococcus xylosus* GM92) were used as starter culture (Kaban and Kaya, 2009). *L. plantarum* GM77 and *S. xylosus* GM92 were grown in MRS (Merck, Darmstadt, Germany) and TSB (Merck, Darmstadt, Germany) broth at 30 °C for 24 h. *L. plantarum* GM77 was inoculated with 10^7 cfu/g, while *S. xylosus* GM92 was inoculated with 10^6 cfu/g into sucuk batters.

Sucuk Production

For the sucuk production, 20 g salt, 10 g garlic, 4 g sucrose, 7 g red pepper, 9 g cumin, 2.5 g allspice, 0.15 g sodium nitrite per 1 kg beef meat and beef fat were used. Considering this basic formulation, 6 sucuk batters (with starter culture) were made using different levels of black pepper (5, 10 or 15 g/kg) and sodium ascorbate (0 or 568 mg/kg). Five kg of sucuk batter per each treatment was prepared in laboratory-type bowl cutter (MADO Typ MTK 662, Dornhan / Schwarzwald). For each treatment, two batches were prepared, thus a total of 12 sucuk batches was obtained.

The sucuk batters were stuffed into collagen casings (Naturin Darm, 38 mm) at 200 ± 10 g using a laboratory-type filling machine (MADO Typ MTK 591, Dornhan / Schwarzwald). The sausages were ripened in an automatic climate unit (Reich, Germany), where temperature, relative humidity and air flow can be controlled automatically. The ripening program was carried out in the automatic climate unit for 2 days with a temperature of 22 ± 1 °C, relative humidity of 90 ± 2%, and on days 3rd and 4th with a temperature of 20 ± 1 °C, relative humidity of 88 ± 2%. On the following days (5th, 6th and 7th days), the temperature was kept constant at 18 ± 1 °C, and the relative humidity was gradually reduced to 80 ± 2%. Air flow was kept at 0.5 m/s in the first 3 days of ripening, and gradually decreased to 0.1 m/s in the following days (Kaban and Kaya, 2009).

Method

After sucuk production, two random samples were taken from each treatment in each production and they were subjected to pH, a_w and volatile compounds analyzes.

Determination of pH and a_w

To determinate to pH value of samples, 10 g of sucuk sample was homogenised with 100 mL distilled water using Ultra-Turrax (T25; IKA-Werke GmbH, Staufen, Germany), then it was measured using a pH meter (Orion 420, Thermo, Waltham, MA).

The water acitivity (a_w) value of samples was determined at 25 °C using an a_w sprint device (Model TH-500; Novasina AG, Lachen, Switzerland). Before the analysis, the instrument was calibrated with 6 different salt solutions.

Analysis of Volatile Compounds

Five g each of the homogenized samples were weighed into 40 mL vials (Supelco, Bellefonte PA, USA). The vials were kept in a thermal block (Supelco, Bellefonte PA, USA) for one hour at 30 °C for the extraction of volatile compounds and it was ensured that volatile compounds were accumulated in the headspace. Then, CAR/PDMS fiber (Supelco, 75 µm, USA) was placed in vials for adsorption of the compounds and kept for 2 hours. After this step, the fiber was injected into the gas chromatography (GC, Agilent Technologies 6890N) device and the compounds were identified by mass spectrometry (MS, Agilent Technologies 5973). Carrier gas used in the system was helium and DB-624 (60 m x 0.250 mm x 1.40 µm, Agilent Tech, USA) was used as column. The oven temperature of the gas chromatography was initially set at 40 °C for 5 min, and then gradually increased to 110 °C with 3 °C/min, 150 °C with 4 °C/min and 210 °C with 10 °C/min, then kept at 210 °C for 12 min. The library of mass spectrometry (NIST, WILEY and FLAVOR) and standard mix (Supelco 44585-U, Bellefonte PA USA) were used for identification (Kaban, 2009).

Statistical Analysis

The use of sodium ascorbate (without or with sodium ascorbate) and black pepper level (5, 10, 15 g/kg) were evaluated as factors. For each treatment, two batters were prepared. The experiments were performed according to the randomised complete block design with two blocks (two replicates). The data was analyzed with two-way ANOVA using the general linear model. The factors were evaluated as fixed effects and replicates as random effect. The means of significant sources of variation were compared using Duncan's multiple comparison test at the $P < 0.05$ level. All statistical analyzes were carried out using the SPSS version 24 statistical program (SPSS Inc., Chicago, IL, USA). Principal component analysis (PCA) was performed with the aid of The Unscrambler software (CAMO software version 10.1).

RESULTS AND DISCUSSION

pH and a_w

The overall effect of sodium ascorbate addition and black pepper level on the pH and a_w of sucuk samples is given Table 1. According to the results, the black pepper level and sodium ascorbate addition did not significantly affect the pH and a_w values of the samples ($P > 0.05$). In this study, starter culture was used in all treatments. Both a_w and pH are hurdle effects for fermented sausages. In sucuk samples, pH and a_w values were below

5.0 and 0.90, respectively (Table 1). Similar results have been reported in previous studies on sucuk made with starter culture (Kaban and Kaya, 2009; Sallan et al., 2019).

Table 1: Mean pH and a_w values for sucuk samples (Mean ± SD)

Treatment	n	pH	a_w
Sodium Ascorbate (Asc)			
without Asc	12	4.86±0.06	0.87±0.04
with Asc	12	4.89±0.05	0.89±0.02
Significance		ns	ns
Black Pepper Level (BP)			
5 g/kg	8	4.90±0.03	0.88±0.01
10 g/kg	8	4.86±0.06	0.89±0.02
15 g/kg	8	4.86±0.08	0.87±0.05
Significance		ns	ns
Asc x BP		ns	ns

ns: not significant, $P > 0.05$; SD: standard deviation.

Volatile Compounds

The overall effects of black pepper level and sodium ascorbate addition on the volatile compound profile of sucuk are given in Table 2. A total of 47 compounds, including 5 aldehydes, 4 aliphatic hydrocarbons, 1 alcohol, 2 aromatic hydrocarbons, 1 acid, 2 ester, 8 sulfur compounds and 24 terpene compounds, were identified in the sucuk samples (Table 2).

Aroma is one of the most important quality attributes in fermented sausages such as sucuk. Compounds formed by biochemical reactions such as lipolysis, proteolysis, lipid oxidation, amino acid catabolism, strecker degradation during fermentation and drying contribute to the aroma (Kaban, 2010; Xiao et al., 2020). The spices in the formulation can also contribute more or less effectively to the aroma (Ordonez et al., 1999).

The use of sodium ascorbate showed no significant effect on aldehydes ($P > 0.05$), while the black pepper reduced the abundance of hexanal and 2-methyl-3 phenyl propanal in sucuk ($P < 0.05$). In fermented sausages, aldehydes can be formed through strecker degradation, carbohydrate metabolism or lipid oxidation (Ordonez et al., 1999). The fact that high black pepper levels (15 g/kg) decrease both hexanal and 2-methyl-3 phenyl propanal levels in sucuk samples in the study may be due to black pepper being a good source of flavonoid and phenolic antioxidants (Zarai et al., 2013). Hexanal is an indicator of lipid oxidation. This compound possesses the typical smell of green grass (Sidira et al., 2015). 2-methyl-3 phenyl propanal is the characteristic aldehyde for sucuk (Kaban, 2010). As in the present study, Stahnke (1999) reported that ascorbate had no effect on aldehydes.

Black pepper had no effect on aliphatic hydrocarbons ($P > 0.05$). Sodium ascorbate, on the other hand, was effective only on hexane ($P < 0.05$). Due to the high threshold values of aliphatic hydrocarbons, their effects on the aroma of fermented sausages are very limited (Ruiz et al., 1999; Ramirez and Cava, 2007). Hexane also had the highest abundance among aliphatic hydrocarbons (Table 2).

Black pepper level did not significantly affect the aromatic hydrocarbons ($P > 0.05$). Similarly, sodium ascorbate had no significant effect on these compounds ($P > 0.05$) (Table 2). In addition, 2 aromatic hydrocarbons were determined in sucuk samples. These compounds were previously determined in a study on sucuk conducted by Kaban (2010).

Ethyl alcohol was the only alcohol that was determined in sucuk samples. The use of ascorbate decreased the level of this compound, while 15 g/kg black pepper level increased ethanol formation ($P < 0.05$). However, there was no statistically significant difference between addition of 5 g/kg and 10 g/kg ($P > 0.05$) (Table 2). Alcohols are generated as reaction products of lipid oxidation, carbohydrate metabolism and amino acid catabolism in dry fermented sausages (Mateo and Zumalacarregui, 1996; Ordonez et al., 1999). Ethyl alcohol determined in sucuk samples was found to be lower in the presence of ascorbate (Table 2). On the other hand, Sallan et al. (2022) reported that the use of ascorbate in a semi-dry fermented sausage has no effect on ethyl alcohol. In a study carried out by Stahnke (1999), it was reported that the addition of ascorbate significantly increases ethanol level. On the other hand, increasing the black pepper level to 15 g/kg increased the ethanol level (Table 2). It is thought that these results are related to the redox potential of the meat fermentation (Huynh et al., 2020). Ordonez et al. (1999) also emphasized that alcohol formation is favored by a greater NADH compared to NAD^+ concentration.

Only acetic acid was determined as acid in sucuk samples (Table 2). Acetic acid is formed through amino acid catabolism and lipid oxidation as well as activities of homofermantative lactic acid bacteria and staphylococci (Montel et al., 1998). This compound, which gives sour note to the product (Montel et al., 1998), was also identified in previous studies on sucuk (Kaban 2010, Demirok Soncu et al., 2020). However, in our study, neither the black pepper content nor the addition of sodium ascorbate significantly affected the acids (P >0.05) (Table 2).

Table 2. Overall effects of ascorbate addition and black pepper levels on the volatile profile of sucuk (means ± SD) (AU x 10^6)

			Ascorbate Addition (Asc)		Black Pepper Level (BP)			*P*	
Compounds	KI	RT	Without Asc	With Asc	5 g/kg	10 g/kg	15 g/kg	Asc	BP
Aldehydes									
Pentanal	742	A	0.38±0.26a	0.37±0.34a	0.52±0.32a	0.24±0.29a	0.35±0.23a	ns	ns
Hexanal	849	A	1.38±0.86a	0.94±0.84a	1.79±0.91a	1.04±0.89ab	0.65±0.25b	ns	*
Octanal	1051	A	0.49±0.25a	1.01±1.36a	1.34±1.58a	0.47±0.27a	0.43±0.15a	ns	ns
Nonanal	1143	A	2.52±1.69a	4.30±6.06a	5.77±7.14a	2.18±1.41a	2.27±1.53a	ns	ns
2-methyl-3-phenyl- propanal	1344	B	9.76±2.49a	10.5±3.16a	11.9±3.15a	10.3±2.44ab	8.23±1.56b	ns	*
Aliphatic Hydrocarbons									
Hexane	600	A	18.2±8.67a	11.3±3.75b	16.6±9.26a	13.70±7.09a	13.93±6.26a	*	ns
Undecane	1100	A	0.56±0.38a	0.62±0.45a	0.72±0.49a	0.58±0.50a	0.47±0.08a	ns	ns
Dodecane	1200	A	2.33±1.44a	1.75±0.79a	1.94±0.90a	2.27±1.22a	1.92±1.44a	ns	ns
Tetradecane	1400	A	2.19±2.33a	1.58±1.57a	1.93±1.87a	2.06±1.61a	1.66±2.27a	ns	ns
Alcohol									
Ethanol	505	A	0.82±0.65a	0.39±0.31b	0.46±0.23b	0.39±0.34b	0.96±0.76a	*	*
Aromatic Hydrocarbons									
Styrene	935	B	0.16±0.22a	0.15±0.13a	0.22±0.22a	0.15±0.18a	0.09±0.09a	ns	ns
1,2-dimethoxy-4-(2-propenyl)- benzene	1434	C	4.94±1.41a	5.65±1.51a	4.79±1.45a	5.65±1.65a	5.45±1.37a	ns	ns
Acid									
Acetic acid	710	A	6.46±2.30a	6.81±3.73a	7.67±3.55a	5.56±2.53a	6.68±2.94a	ns	ns
Esters									
Methyl acetate	890	C	1.01±0.43a	0.69±0.44a	1.09±0.51a	0.72±0.43a	0.74±0.39a	ns	ns
Hexyl butyrate	1215	B	1.43±1.03a	1.00±0.50a	1.55±0.96a	1.41±0.85ab	0.68±0.28b	ns	*
Sulfur Compounds									
Allyl mercaptan	574	B	18.6±8.60a	13.7±6.99a	19.43±9.3a	12.26±5.47a	16.62±8.18a	ns	ns
Allyl methyl sulfide	730	B	4.21±2.45a	2.33±1.56b	4.39±2.89a	2.54±1.74a	2.87±1.64a	*	ns
1-(methylthio)- 1-propene	832	C	0.36±0.20a	0.36±0.26a	0.52±0.21a	0.19±0.18b	0.36±0.18ab	ns	*
Dimethyl disulfide	834	B	0.98±0.43a	0.71±0.37a	1.15±0.45a	0.71±0.38b	0.67±0.23b	ns	*
3,3'-thiobis-1-propene	888	B	3.01±1.88a	1.25±0.77b	2.96±2.05a	1.78±1.72a	1.63±0.87a	**	ns

Table 2. continue

			Ascorbate Addition (Asc)		Black Pepper Level (BP)			*P*	
Compounds	KI	RT	Without Asc	With Asc	5 g/kg	10 g/kg	15 g/kg	Asc	BP
Methyl 2-propenyl disulfide	969	C	3.46±1.74a	2.30±1.15b	3.98±2.00a	2.25±1.03b	2.43±0.92b	*	*
Methyl trans-propenyl-disulfide	977	B	0.46±0.24a	0.48±0.23a	0.63±0.22a	0.36±0.26b	0.43±0.11ab	ns	ns
Di-2-propenyl- disulfide	1135	B	10.28±4.1a	7.22±2.29b	11.31±4.8a	7.23±1.96b	7.69±2.11b	*	*
Terpenes									
α-Thujene	944	B	3.15±1.28a	3.35±1.42a	2.51±0.40b	2.50±1.14b	4.73±0.71a	ns	**
α -Pinene	951	C	4.61±1.79a	4.86±2.19a	2.94±0.52c	4.18±1.01b	7.08±1.04a	ns	**
Camphene	970	C	0.38±0.21a	0.48±0.23a	0.49±0.29a	0.41±0.23a	0.38±0.12a	ns	ns
β-Pinene	996	C	25.6±5.99a	26.4±7.26a	27.23±7.8a	24.13±6.38a	27.84±7.89a	ns	ns
α -Phellandrene	1019	C	5.48±2.95a	5.59±3.25a	3.53±0.33b	4.08±2.56b	9.00±1.70a	ns	**
3-Carene	1026	C	5.63±2.13a	5.14±2.04a	3.06±0.45c	5.91±1.64b	7.18±0.91a	ns	**
α-Terpinene	1042	C	2.33±0.65a	2.50±0.71a	2.16±0.48b	2.11±0.70b	2.97±0.46a	ns	*
D-Limonene	1054	A	21.76±7.4a	19.5±10.8a	14.39±3.6b	16.92±5.35b	30.59±7.6a	ns	**
β-Phellandrene	1060	C	4.39±1.61a	4.30±1.96a	3.38±0.70b	3.43±1.34b	6.23±1.32a	ns	**
Cis-Ocimene	1097	C	2.42±0.74a	2.54±0.59a	2.75±0.89a	2.24±0.39a	2.47±0.56a	ns	ns
γ-Terpinene	1099	C	30.20±8.5a	28.16±5.2a	33.2±7.59a	26.36±6.14a	27.95±5.98a	ns	ns
Terpinolene	1104	C	1.77±0.43a	1.17±0.42a	1.11±0.55a	1.02±0.34a	1.38±0.25a	ns	ns
Linalool	1161	A	6.94±2.86a	6.06±1.50a	7.25±2.64a	6.47±2.52a	5.77±1.58a	ns	ns
4-Terpinenol	1235	B	3.25±1.09a	2.87±0.91a	2.82±1.19a	2.83±0.58a	3.53±1.11a	ns	ns
α -Terpineol	1258	C	2.44±1.51a	2.48±0.81a	2.54±0.65a	2.68±1.95a	2.16±0.46a	ns	ns
Delta elemene	1391	C	2.36±0.78a	2.29±0.76a	1.83±0.50a	2.46±0.63ab	2.69±0.88b	ns	ns
2-caren-10-al	1393	C	1.88±1.00a	1.55±0.95a	2.06±0.56a	1.86±1.29a	1.23±0.82a	ns	ns
α -Cubebene	1396	C	0.87±0.57a	0.88±0.28a	0.80±0.18a	0.76±0.56a	1.05±0.48a	ns	ns
Copaene	1433	C	5.76±2.73a	6.19±2.11a	4.44±0.30b	5.27±2.85b	8.22±1.28a	ns	**
Eugenol	1456	B	3.67±1.27a	3.79±1.37a	3.30±0.71a	4.15±1.45a	3.74±1.56a	ns	ns
Trans-Caryophyllene	1468	B	2.02±0.73a	1.64±0.64a	1.56±0.39b	1.49±0.66b	2.43±0.64a	ns	**
α -trans- Bergamotene	1472	C	2.87±1.53a	3.06±1.67a	1.81±0.60b	2.77±1.75b	4.32±0.99a	ns	**
Caryophyllene	1490	B	22.2±12.9a	24.7±13.6a	14.2±3.89b	20.7±14.84b	35.44±7.2a	ns	**
α -Caryophyllene	1504	B	1.97±1.09a	2.26±1.19a	1.30±0.41b	1.99±1.30b	3.04±0.74a	ns	**

P: statistical significance; ns: not significant $P > 0.05$; *: $P < 0.05$; **: $P < 0.01$. Results are expressed in Arbitrary Area Units (× 10^6); RI: Reliability of identification; A: mass spectrum and retention time identical with an authentic sample; B: mass spectrum and Kovats index from literature in accordance; C: tentative identification by mass spectrum; KI: Kovats index calculated for DB-624 capillary column (60 m × 0.25 mm × 1.4 µm) installed on a gas chromatograph equipped with a mass selective detector. Different letters (a-c) indicate significant differences between the levels in the groups.

Another volatile chemical group identified in sucuk is esters. The esters determined were hexyl butyrate and methyl acetate. None of the compounds were affected by the use of ascorbate. The black pepper level of 15 g/kg showed lower abundance for hexyl butyrate than for 5g/kg ($P > 0.05$) (Table 2). Esters have low threshold values and give a fruity note to dry fermented sausages. The presence of alcohols and acids, as well as the esterification activity of staphylococci play an important role in ester formation (Xiao et al., 2020).

Among the sulfur compounds, allyl mercaptan showed a higher abundance in sucuk samples. However, this compound was not influenced by the addition of ascorbate ($P > 0.05$). The use of ascorbate affected the levels of 3,3-thiobis-1-propene, allyl methyl sulfide, methyl-2-propenyl-disulfide, di-2-propenyl-disulfide significantly ($P < 0.05$). The use of ascorbate in sucuk production reduced the abundance of some sulfur compounds (Table 2). Sulfur compounds can be formed by enzymatic reactions (allium types such as onion and garlic or vegetables such as broccoli, cabbage and cauliflower) or by heat treatment applications. Due to their low aroma and taste thresholds, volatile sulfur compounds can exhibit sensory potential at low concentrations (McGorrin, 2011). The black pepper level was also significantly effective on 1- (methylthio)-propene, dimethyl disulfide, methyl 2-propenyl-disulfide and di-2-propenyl-disulfide compounds ($P < 0.05$). Increasing the level of black pepper in sucuk production from 5 g/kg to 10 g/kg led to a decrease in the abundance of 1- (methylthio) - 1-propene, dimethyl disulfide, methyl 2-propenyl-disulfide and di-2-propenyl-disulfide compounds. However, increasing the black pepper level to 15 g/kg caused no significant difference ($P > 0.05$).

The use of ascorbate had no significant effect on terpenes in sucuk samples ($P > 0.05$). On the other hand, the black pepper level was effective on 12 of 24 terpenes at the level of $P < 0.05$ or $P < 0.01$ (Table 2). Terpenes play an important role in the volatile profile of sucuk. In addition to black pepper, many spices such as garlic, red pepper, allspice and cumin are used in the sucuk (Kaban, 2010). The main source of terpenes are spices (Flores, 2018). It is reported that the main volatile compounds of black pepper are caryophyllene, D-limonene, 3-carene (Li et al., 2020). In addition, volatile compounds such as alpha-pinene, sabinene, 1-alpha-pellandrene are included in the volatile profile of black pepper (Chi and Wu, 2007; Lilie et al., 2007; Munekata et al., 2020). However, volatile profile of black pepper varies depending on the many factors such as chemo-types, geographic origin, seasonality, and use of oil extraction methods (Li et al., 2020). In the present study, caryophyllene, D-limonene, and γ-terpinene were the most volatile compounds with the highest abundance among the terpenes in sucuk samples. Of these compounds, γ-Terpinene was not affected by the black pepper level ($P > 0.05$). On the other hand, caryophyllene and D-limonene, which are of the main volatile compounds of black pepper, showed higher abundance at 15 g/kg black pepper level (Table 2). 3-Carene, a bicyclic monoterpene, increased with increasing black pepper level. When the black pepper level was increased from 5 g/kg to 15 g/kg, the abundance of this compound increased approximately 2.5 times (Table 2). α-pinene also increased significantly as black pepper levels increased. In sucuk samples with 5 g/kg black pepper and sucuk samples with 10 g/kg black pepper, it was found that there were no significant differences in terms of α-thujene, alpha-phellandrene, α-terpinene, D-limonene, beta-phellandrene, copaene, trans-caryophyllene, alpha-trans-bergamotene, caryophyllene, alpha-caryophyllene (Table 2). The black pepper content of 15 g/kg showed a higher abundance for these compounds than for 5 and 10 g/kg.

Principal Component Analysis (PCA)

PCA was performed to evaluate the relationships between factors (ascorbate addition and black pepper level) and volatile compounds. The eigenvalues, variances and their cumulative proportions are shown in Table 3. The eigenvectors of the first three principal components (PC) are given in Table 4. The first three principal components (PC1, PC2 and PC3) were analyzed having eigenvalues greater than 1.0; explaining 78.40, 17.90 and 3.70% of the variance, respectively. The first two principal components explained 96.30% of the total variance (Table 3). It was found that D-limonene and caryophyllene were the most related variable for PC1; allyl mercaptane, γ-terpinene, D-limonene, hexane for PC2 (Table 4).

Table 3. Eigenvalue scores, variances and their cumulative proportions of first three components

Principal component	Eigenvalue		Explained variance	
	For PC	Cumulative	For PC	Cumulative
PC1	119.00	119.00	78.40	78.40
PC2	27.23	146.23	17.90	96.30
PC3	5.57	151.80	3.70	100.00

Table 4. Eigenvectors of the variance–covariance matrix used for PCA

Variable	PC1	PC2	PC3
Pentanal	-0.004	0.014	0.023
Hexanal	-0.036	0.039	-0.003
Octanal	-0.025	0.011	0.129
Nonanal	-0.093	0.066	0.491
2-methyl-3-phenyl-propanal	-0.116	-0.020	0.159
Hexane	-0.087	0.406	-0.578
Undecane	-0.007	0.001	0.014
Dodecane	-0.004	0.003	-0.101
Tetradecane	-0.012	0.011	-0.093
Ethanol	0.018	0.033	-0.029
Styrene	0.004	0.002	0.003
1,2-dimethoxy-4-(2-propenyl)-benzene	0.018	-0.067	0.010
Acetic acid	-0.018	0.092	0.233
Methyl acetate	-0.010	0.028	-0.008
Hexyl butyrate	-0.030	0.007	-0.058
Allyl mercaptan	-0.056	0.578	0.096
Allyl methyl sulfide	-0.044	0.157	-0.068
1-(methylthio) -1-propene	-0.004	0.016	0.030
Dimethyl disulfide	-0.015	0.028	0.002
3,3-thiobis-1-Propene	-0.042	0.116	-0.117
Methyl 2-propenyl-disulfide	-0.044	0.120	0.002
Methyl trans-propenyl-disulfide	-0.005	0.011	0.025
Di-2-propenyl-disulfide	-0.104	0.295	-0.029
α -thujene	0.079	0.042	0.080
α-pinene	0.138	0.006	0.005
Camphene	-0.003	-0.002	0.017
B-pinene	0.042	0.269	0.149
α-phellandrene	0.190	0.084	0.093
3-Carene	0.123	-0.060	-0.247
α-Terpinene	0.029	0.014	0.046
D-Limonene	0.551	0.302	-0.116
β-phellandrene	0.100	0.064	0.057
Cis-ocimene	-0.006	0.020	0.058
γ-Terpinene	-0.139	0.385	0.336
Terpinolene	0.010	0.012	0.016
Linalool	-0.048	0.052	-0.060
4-Terpinenol	0.025	0.032	-0.029
α -Terpineol	-0.015	-0.019	-0.020

Table 2. continue

Variable	PC1	PC2	PC3
Delta elemene	0.026	-0.016	-0.050
2-caren-10-al	-0.028	0.008	-0.040
α - cubebene	0.009	0.008	0.013
Copaene	0.129	0.015	0.060
Eugenol	0.009	-0.048	-0.062
Trans-caryophyllene	0.030	0.040	-0.017
α -Trans-bergamotene	0.082	-0.011	-0.012
Caryophyllene	0.709	-0.026	0.153
α caryophyllene	0.057	-0.016	0.006

PC1 was positively related with sucuk containing 15 g/kg black pepper and with ascorbate. In addition, PC1 was inversely related with the levels of 5 g/kg black pepper and 10 g/kg black pepper as well as without ascorbate (Fig. 1). At the same time, PC1 separated the groups with ascorbate and without ascorbate well. On the other hand, sucuk samples with 5 g/kg black pepper, 15 g/kg black pepper and sucuk samples without ascorbate placed on positive side of PC2. PC2 showed that 10 g/kg black pepper correlated more strongly with ascorbate than other black pepper levels, and placed on negative side for PC2 (Fig. 1).

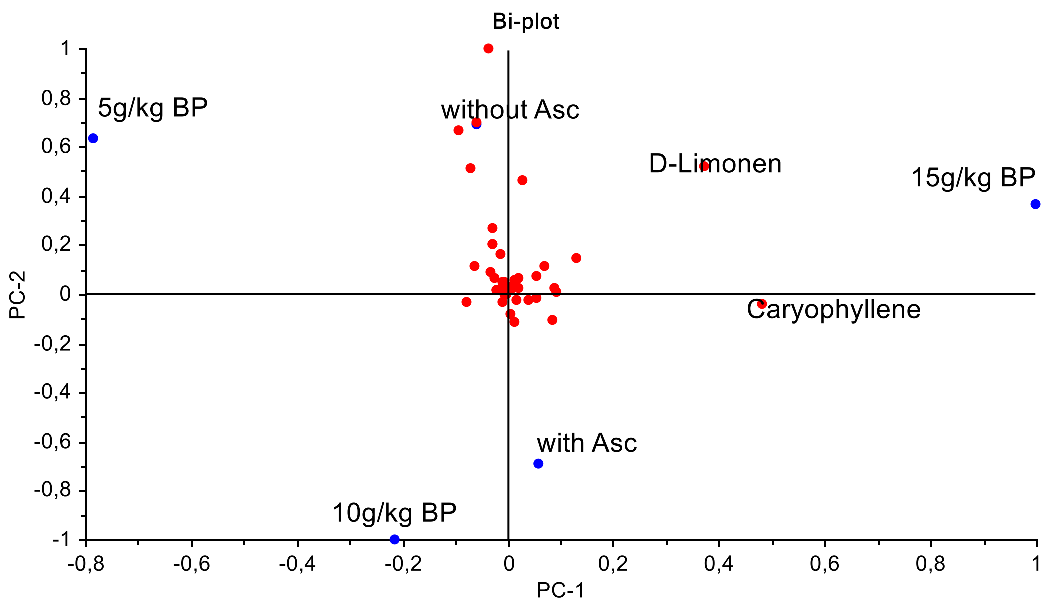

Figure 1: Principal component analysis biplot of the relationships between factors (sodium ascorbate addition and black pepper level) and volatile compounds

CONCLUSION

It is a notable finding from the study that ascorbate has no effect on aldehydes, which are mainly formed by lipid oxidation. Increasing the level of black pepper also resulted a decrease in the abundance of sulfur compounds. While the black pepper levels reduced hexanal levels, which is an indicator of lipid oxidation, it raised ethanol levels. Terpenes were the main group, in which black pepper is effective. An increase in the abundance of many terpene compounds has been observed with increasing black pepper level. The group with 15 g/kg black pepper placed on the positive side of PC1, while the groups with 5 and

10 g/kg black pepper placed on the negative side of PC1. The sucuk samples containing 15 g/kg black pepper had a high relationship with D-limonene and caryophyllene. In addition, some volatile compounds were affected by black pepper levels and showed different dimensions. Moreover, the use of ascorbate also affected the dimensions of some volatile compounds.

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DECLARATİON OF COMPETİNG INTEREST
The authors have declared no conflict of interest.

AUTHORS' CONTRIBUTIONS
Selen SALLAN performed investigation, formal analysis and writing–original draft. Güzin KABAN did methodology, validation and writing. Mükerrem KAYA was supervisior and administrated the project, performed writing – review & editing.

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