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Effects of Autochthonous Starter Cultures on the Quality Characteristics of Traditionally Produced Sucuk

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Abstract

This study aimed to evaluate the impact of autochthonous strains (*Pediococcus pentosaceus* 128b, *Latilactobacillus sakei* S15, *Lactiplantibacillus plantarum* S91, *L. plantarum* S24 and *Staphylococcus carnosus* G109) used as mono and mixed starter cultures on the quality attributes of traditionally produced sucuk, a Turkish dry fermented sausage, in a local small-scale facility. At the end of ripening, samples underwent comprehensive microbiological and physicochemical analyses. The use of autochthonous starter cultures (ASC) showed no statistically significant influence on thiobarbituric acid-reactive substances and water activity value. Lower pH values were observed in groups with autochthonous lactic acid bacteria strains (ALABS) compared to the control group. However, ALABS inhibited *Micrococcus/Staphylococcus* growth by rapidly lowering the pH, except in the groups with *S. carnosus* G109. The use of ASC led to an increase in the L^* values of sucuk samples, except monoculture *L. plantarum* S91. The correlation heat map illustrating the relationships between the starter cultures and volatile compounds revealed that all groups containing *L. plantarum* S91 exhibit a volatile compound profile different from other single or mixed cultures. According to the results of the principal component analysis performed to determine the relationship between the chemical groups of the starter cultures and volatile compounds, the groups containing *L. plantarum* S91 differed from the other groups and showed positive correlations with phenols, furans, acids, terpenes, aromatic hydrocarbons, ketones, nitrogenous compounds, esters, and aliphatic hydrocarbons.

Keywords: fermented sausage; lactic acid bacteria; *Staphylococcus*; volatile compounds; quality properties

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1. Introduction

Fermentation is one of the oldest known methods of food preservation. This method relies on the biological activities of microorganisms. Metabolites formed during fermentation suppress the growth and viability of food-borne pathogens and spoilage microorganisms in foodstuffs [1]. Meat fermentation is defined as a microbial ecosystem in which bacteria, yeasts, and molds coexist. During fermentation, perishable substances such as meat and fat are improved adding sodium chloride, and through the effectiveness of the drying process are transformed into microbiologically stable final products [2]. Fermented sausages are gastronomically valuable products with a long history [3]. Fermentation plays a fundamental role in the production of these products. Although fermentation is performed

by spontaneous lactic acid bacteria (LAB) in traditional production, starter cultures are commonly employed in industrial production. Choosing a starter culture suitable for the product type is a key production strategy [4]. Fermented sausages are produced by adding salt, sugars, nitrite, nitrate, and spices to a mixture of minced fat and lean meat, filling the batter into casings, and keeping the products in ripening chambers for several weeks, where they are hung vertically [5]. Traditional foods are important cultural heritage products that bridge a country's past and present [6]. One of them is sucuk, the best-known traditional dry fermented meat product in Türkiye, made using beef and/or water buffalo meat, beef fat and/or sheep tail fat, sugar, salt, curing agents (nitrite and/or nitrate), and many spices. An important feature that distinguishes sucuk from other European sausages is that it is not smoked. Sucuk is prepared by filling small-diameter casings and subjecting them to a relatively short ripening process [7].

Food fermentation is a process driven by the adventitious microbiota naturally found in raw food ingredients and in the environment and has evolved over centuries towards the controlled use of starter cultures [8]. The choice of starter culture determines the characteristics and quality of fermented sausages. Starter cultures need to be well adapted and dominant in the meat environment and throughout the production process. Commercial starter cultures, which are widely used in fermented sausage production, are selected according to their fermentative, proteolytic, and lipolytic properties, but they may not always be preferred because they can cause the loss of some desirable sensory properties [9]. The addition of desirable microorganisms in the meat industry is used to ensure product safety by inhibiting pathogenic bacteria and prolonging the shelf life of the product by preventing undesirable changes caused by spoilage microorganisms or abiotic reactions, while also providing new sensory properties and beneficial health effects [10]. LAB and coagulase-negative staphylococci (CoNS) are the two main bacterial groups isolated from dry fermented sausages [11]. LAB decrease pH by producing lactic acid, thereby preventing the growth of undesirable bacteria and affecting protein coagulation. CoNS possess nitrate reductase and catalase activities [6]. Thus, they play a role in color formation and delaying lipid oxidation. They also contribute to flavor formation via lipolytic and proteolytic activities [12]. The use of autochthonous LAB originating from fermented meats offers several advantages such as better adaptation to meat fermentation conditions, control of the ripening process, and inhibition of the growth of spontaneous microorganisms [6,11,13].

Autochthonous starter cultures (ASC) can be used to produce typical regional fermented meat products, ensure new sensory properties, and improve product safety, stability, and shelf life [14]. Several studies have been conducted on the use of autochthonous strains as starter cultures for sucuk production [9,15–20]. These studies were conducted under controlled fermentation conditions. However, to the best of our knowledge, no studies have investigated the applicability of autochthonous strains under small-scale facility conditions in the traditional production of sucuk. Traditional fermented sausage production often relies on the spontaneous microorganisms present in the raw materials and the production environment, resulting in an uncontrolled process. Unpredictable changes in the microbiota can occur even within the same batch, leading to inconsistencies in product flavor and safety. Consequently, ensuring uniformity and maintaining high quality in sausages can be challenging. The best solution to this problem is the selection and inoculation of appropriate starter cultures [21,22]. On the other hand, the use of mixed starter cultures consisting of lactic acid bacteria (LAB) and coagulase-negative staphylococci (CNS) is also recommended to achieve the desired characteristics in fermented sausages. Moreover, it is emphasized that the bacterial communities of fermented sausages have significant effects on their aroma and taste properties [21–23]. Therefore, this study aimed to evaluate the effects of selected autochthonous strains (*Latilactobacillus sakei* S15, *Lactiplantibacillus*

plantarum S24, *L. plantarum* S91, *Pediococcus pentosaceus* 128b, and *Staphylococcus carnosus* G109) previously isolated and identified from sucuk [24], when applied as mono and mixed starter cultures under traditional processing conditions. For this purpose, ten different sucuk formulations, including a control group, were prepared and produced in a local small-scale facility using traditional techniques. Following ripening, the samples were subjected to comprehensive microbiological and physicochemical analyses to determine the influence of these starter cultures on the overall quality characteristics of traditionally produced sucuk.

2. Materials and Methods

2.1. Materials

In this study, beef cuts from the round and chuck regions of the carcass were trimmed into smaller pieces and stored at −20 °C. Beef fat (intermuscular fat) was also obtained from the beef carcass, cut into small pieces, and stored at −20 °C.

Pediococcus pentosaceus 128b (KT327865), *Latilactobacillus sakei* S15 (KR025387), *Lactiplantibacillus plantarum* S91 (KT327838), *L. plantarum* S24 (KT327849), and *Staphylococcus carnosus* G109 (KY011911), isolated and identified through 16S rRNA gene sequencing from sucuk, were used as starter cultures [24]. *L. sakei* S15, *L. plantarum* S24, *L. plantarum* S91 and *P. pentosaceus* 128b were grown on de Man, Rogosa and Sharpe broth (Merck, Darmstadt, Germany), whereas *S. carnosus* G109 was grown in Brain Heart Infusion broth (Merck, Darmstadt, Germany) at 30 °C for 24 h.

2.2. Production of Sucuk

Sucuk production was carried out in a local small-scale facility using traditional techniques. Sucuk batter was prepared using, per kilogram of meat and fat mixture (80% lean beef and 20% beef fat), 25 g salt, 4 g sucrose, 7 g red pepper, 5 g black pepper, 9 g cumin, 2.5 g allspice, and 10 g garlic. Nine different starter culture formulations were prepared, as presented in Table 1. *P. pentosaceus* 128b, *L. sakei* S15, *L. plantarum* S24 and *L. plantarum* S91 were added to the sucuk batter at 7 log cfu/g, and *S. carnosus* G109 was added at 6 log cfu/g. Batches produced without starter cultures were used as the control group. After the sucuk batter was prepared, it was stuffed into collagen casings (38 mm, Naturin Darm, Germany) and ripened in a climate chamber (Özlü Makine, Zonguldak, Türkiye) (fermentation at 24 ± 1 °C for 24 h, followed by drying at 18 ± 2 °C for 7 days) under plant conditions.

Table 1. Design of mono and mixed starter cultures.

Strains	Starter Culture/s	Strains	Starter Culture/s
Control	-	E	*L. plantarum* S24 + *L. plantarum* S91 + *L. sakei* S15
A	*L. sakei* S15	F	*P. pentosaceus* S128b + *L. plantarum* S91 + *L. sakei* S15
B	*L. plantarum* S91	G	*L. sakei* S15 + *S. carnosus* G109
C	*P. pentosaceus* 128b	H	*L. sakei* S15 + *L. plantarum* S91 + *S. carnosus* G109
D	*L. plantarum* S91 + *L. sakei* S15	I	*P. pentosaceus* S128b + *S. carnosus* G109 + *L. sakei* S15

2.3. Microbiological Analysis

Each 25 g sample portion was aseptically weighed, transferred into a sterile stomacher bag, and diluted with 225 mL of sterile physiological saline solution (0.85%). The mixture was homogenized for 2 min using a stomacher (Lab Stomacher Blender 400-BA 7021, Seward, UK), and the spread-plate method was used. Lactic acid bacteria counts were determined on de Man, Rogosa, and Sharpe agar (MRS, Merck, Darmstadt, Germany) under anaerobic conditions (Anaerocult A, Merck, Darmstadt, Germany) at 30 °C for

48 h. *Micrococcus/Staphylococcus* were enumerated on Mannitol Salt Phenol Red agar (MSA, Merck, Darmstadt, Germany) incubated at 30 °C for 48 h. Mold/yeast counts were quantified using Rose Bengal Chloramphenicol agar (RBC, Merck, Darmstadt, Germany) incubated at 25 °C for 72 h. *Enterobacteriaceae* were enumerated on Violet Red Bile Dextrose agar (VRBD, Merck, Darmstadt, Germany) incubated at 30 °C for 48 h [25]. All microbial counts were reported as log10 colony-forming unit per g (log10 cfu/g).

2.4. pH and Water Activity (a_w)

For pH analysis, 10 g of each sample was homogenized with 100 mL of distilled water (1:10 *w*/*v*) using an Ultra-Turrax homogenizer (IKA T25 basic, Staufen, Germany). The pH was subsequently measured with a calibrated digital pH meter (S220, Mettler-Toledo, Switzerland). Water activity (a_w) was determined using a TH-500 a_w Sprint apparatus (Novasina, Pfäffikon, Switzerland), with samples placed in the measuring chamber and analyzed at a constant temperature of 25 °C.

2.5. Thiobarbituric Acid Reactive Substances (TBARS)

The TBARS values were determined according to the method described by Lemon [26], and the results were expressed as mg of malondialdehyde (MDA) per kg of sample.

2.6. Instrumental Color

The color parameters of the sucuk samples were evaluated using a Minolta colorimeter (CR-200, Minolta Co., Osaka, Japan). Color measurements were recorded according to the CIELAB color system as defined by the International Commission on Illumination (CIE), using the *L**, *a**, and *b** coordinates [20]. In this system, *L** represents sample lightness, *a** indicates the degree of redness, and *b** denotes the level of yellowness, reflecting a three-dimensional model of color assessment.

2.7. Volatile Compound Analysis

The method described by Kaban and Kaya [16] was employed for the analysis of volatile compounds in sucuk samples. Headspace volatile compounds were extracted using a solid-phase microextraction (SPME) device (Supelco, Bellefonte, PA, USA). A 75 µm carboxen/polydimethylsiloxane (CAR/PDMS) fiber was utilized and placed in sealed vials for 2 h to allow adsorption of volatiles. Following extraction, the fiber was introduced into a gas chromatograph (GC; Agilent Technologies 6890N) in splitless mode, with injection at 250 °C for 6 min. The GC was coupled with a mass-selective detector (MS; Agilent Technologies 5973) to identify volatile compounds. Separation was achieved using a DB-624 capillary column (Agilent J&W Scientific, Santa Clara, CA, USA; 60 m length, 0.25 mm i.d., 1.4 µm film thickness). Helium was used as the carrier gas at a flow rate of 1 mL/min. The oven temperature program commenced at 40 °C (held for 5 min), followed by a gradual increase to 110 °C at 3 °C/min, to 150 °C at 4 °C/min, and finally to 210 °C at 10 °C/min, where it was held for 12 min. The total analysis time was 56.3 min, and the GC-MS interface temperature was maintained at 280 °C. Mass spectra were obtained using electron impact ionization at 70 eV, covering a mass range of 30–400 amu.

Compound identification was carried out by comparing the mass spectra with databases from the NIST, Flavor and Wiley, as well as by matching retention indices with those reported in the literature. Kovats indices were calculated using a reference standard (Supelco 44585-U, Bellefonte, PA, USA). The results were expressed as arbitrary units of area (AU $\times$ 10^6) and presented as mean values from three independent replicates.

2.8. Statistical Analysis

Two independent manufacturing processes were carried out for each treatment on different days (two batters per treatment). Two random samples were taken from each treatment in each production for the analyses. The data obtained from analyses related to the use of starter cultures were statistically evaluated using a completely randomized design with two replicates. Differences between mean values were assessed using Duncan's multiple range test, performed with SPSS version 24 software (SPSS Inc., Chicago, IL, USA). To determine relationship between the starter cultures and volatile compounds, the correlation heatmap was applied using ChiPlot [27]. To determine the relationship between the starter cultures and chemical groups of volatile compounds, PCA was carried out using the Minitab 17.1.0 software (State College, PA, USA).

3. Results and Discussion

3.1. Microbiological Results

The microbiological count results of sucuk produced with selected autochthonous starter cultures (ASCs) are shown in Figure 1. In the control group, spontaneous LAB exhibited good growth, and the addition of ASCs had no significant effect on LAB counts. Accordingly, the count of LAB was generally 8 log cfu/g at the end of the ripening period. The LAB strains used in this study showed good growth and quickly became dominant over time. These results indicate that spontaneous lactic acid bacteria exhibited good growth. Nevertheless, the composition of spontaneous microbiota is highly variable and influenced by numerous factors, making controlled fermentation inherently unpredictable. Therefore, the use of starter cultures is essential to ensure uniformity and maintain high product quality in sausages [10,21,22,28]. The main role of LAB in meat fermentation is to grow and survive under production conditions and to cause a rapid decrease in pH in the batter [29]. This result is consistent with those previously reported by Kaban and Kaya [15,30], Kaban et al. [19], and Akköse et al. [20].

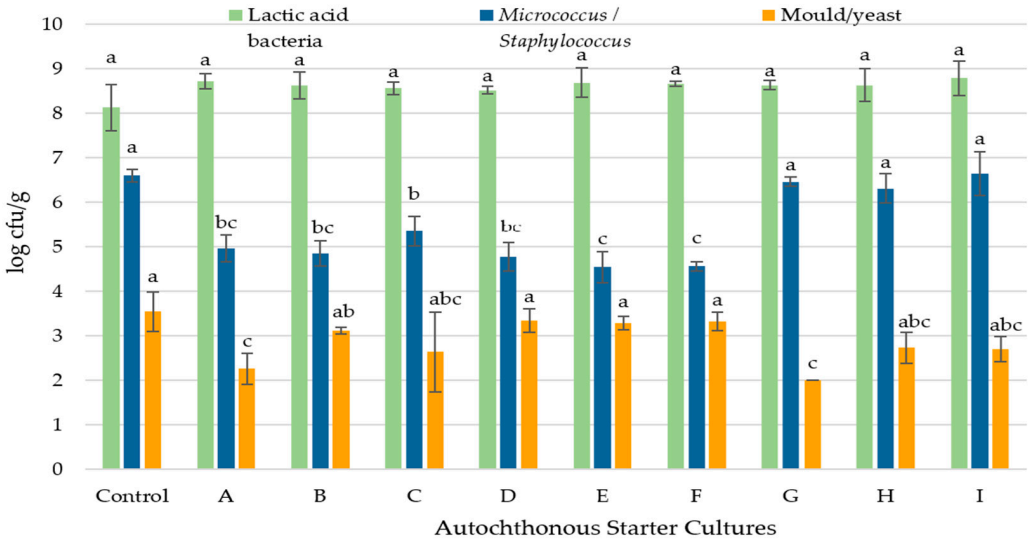


Figure 1. The effects of starter cultures on microbiological properties of sucuk samples (Control: without starter culture, A: *L. sakei* S15, B: *L. plantarum* S91, C: *P. pentosaceus* 128b, D: *L. plantarum* S91 + *L. sakei* S15, E: *L. plantarum* S24 + *L. plantarum* S91 + *L. sakei* S15, F: *P. pentosaceus* S128b + *L. plantarum* S91 + *L. sakei* S15, G: *L. sakei* S15 + *S. carnosus* G109, H: *L. sakei* S15 + *L. plantarum* S91 + *S. carnosus* G109, I: *P. pentosaceus* S128b + *S. carnosus* G109 + *L. sakei* S15). (a–c: Different lowercase letters indicate significant differences between autochthonous starter culture for each microbiological properties) ($p < 0.05$).

The use of ASC had a significant effect on the count of *Micrococcus/Staphylococcus* ($p < 0.01$). According to Duncan's multiple range test, there was no statistical difference between the control group and the groups containing *S. carnosus* G109 in terms of *Micrococcus/Staphylococcus* (Figure 1). Due to the slow decrease in pH in the control group, spontaneous *Micrococcus/Staphylococcus* grew and reached 6 log cfu/g. In contrast, in the groups (A, B, C, D, E and F) using only autochthonous lactic acid bacteria strains (ALABS) as starter cultures, spontaneous *Micrococcus/Staphylococcus* counts remained lower due to rapid acidification (Figure 1). These results are due to the fact that *Micrococcus/Staphylococcus* are acid-sensitive microorganisms [16,30,31]. In the groups (G, H and I) containing both ALABS and *S. carnosus* G109, however, *S. carnosus* G109 maintained its viability and even exhibited slight growth despite the rapid acidification. Therefore, the *Micrococcus/Staphylococcus* count was higher in these groups compared to the other groups, except for the control group (Figure 1). These results show that *S. carnosus* G109 is a good starter culture candidate for sucuk. Corbiere et al. [32] found that *S. carnosus* survived under plant conditions in dry fermented sausages. In other studies on sucuk, it was reported that *S. carnosus* used as a starter culture significantly preserved its viability throughout the process, with *Micrococcus/Staphylococcus* counts of approximately 6 log cfu/g [19,30].

Enterobacteriaceae counts were below the detectable limit (<2 log cfu/g) during ripening in all sucuk groups produced with different starter cultures. The growth of *Enterobacteriaceae* is promoted by high initial water activity and pH, low concentrations of fermentable carbohydrates, low numbers of lactobacilli in the fresh sausage mixture, the absence of nitrate or nitrite, and sometimes insufficient ripening temperatures [33]. The fact that *Enterobacteriaceae* counts were below the detectable limit even in sausage batter indicates the hygienic quality of the raw material. Because bacteria belonging to the *Enterobacteriaceae* family are sensitive to low pH (<5.3) and a_w (<0.96) values, their numbers decrease during ripening [20]. Casquete et al. [34] emphasized that the addition of autochthonous starter cultures reduced the growth of *Enterobacteriaceae* at the beginning of the ripening process. Casaburi et al. [35] reported that after a 3-day ripening period, *Enterobacteriaceae* counts increased by approximately 2 log in the control sausage samples, whereas a decrease was observed in sausages with added starter cultures. This situation can be explained by the rapid decrease in pH following the addition of starter cultures. Bonomo et al. [5] reported that *Enterobacteriaceae* could not be detected in sausage samples containing ASC at the end of ripening. Similar results have been reported by Dominguez et al. [36].

The mold/yeast counts of the samples were found to be 10^2–10^3 cfu/g. Yeast/mold counts were significantly affected by the addition of starter cultures ($p < 0.05$). Groups A and G had lower yeast/mold counts than the control group (Figure 1). The yeast/mold counts of the other groups did not differ from those of the control group. Similarly, Palavecino Prpich et al. [37] reported that yeast/mold counts were not different from those of the control group.

3.2. pH, a_w, and TBARS Values

The pH, a_w, and TBARS values of the sucuk samples produced using ASC are shown in Table 2. The pH of the sucuk samples was very significantly affected by the addition of ASC ($p < 0.01$). The pH value, which was 5.53 in the control group, was found to be below 5.0 in all other groups (Table 2). Moreover, our findings were in good agreement with those of other researchers [36,38–40], who reported lower pH values in samples inoculated with starter cultures compared to the control. In the current study, groups B, D, E, F, and H, which used *L. plantarum* as a monoculture or mixed culture, exhibited statistically lower pH values than groups using other starter cultures. These results also indicate that *L. plantarum* S91 is better adapted to the environment of sucuk fermentation. On the other

hand, Pragalaki et al. [41] determined that the separate use of autochthonous *L. sakei* 8416 and *L. sakei* 4413 strains did not affect the pH level. In dry fermented sausages such as sucuk, salami, and Rohwurst, the water-holding capacity of meat proteins decreases as the pH drops to around 5.3 during fermentation, thereby accelerating the drying of the product. With drying, part of the water is removed, and thus the a_w value decreases. Acidification that occurs during fermentation and the consequent decrease in pH are extremely important for the development of color, flavor, and texture in meat products. In addition, a decrease in pH is important not only for the inhibition of spoilage microorganisms but also for the prevention of pathogenic microorganism growth [28].

Table 2. The effects of starter cultures on pH, a_w and TBARS values of sucuk samples (mean ± standard deviation).

Starter Cultures	pH	a_w	TBARS (mg MDA/kg)
Control	5.53 ± 0.24 [a]	0.899 ± 0.00 [a]	0.75 ± 0.18 [a]
A	4.89 ± 0.29 [b]	0.897 ± 0.01 [a]	0.53 ± 0.19 [a]
B	4.67 ± 0.18 [c]	0.899 ± 0.00 [a]	0.60 ± 0.37 [a]
C	4.86 ± 0.26 [b]	0.901 ± 0.01 [a]	0.88 ± 0.57 [a]
D	4.65 ± 0.13 [c]	0.898 ± 0.00 [a]	0.64 ± 0.35 [a]
E	4.64 ± 0.18 [c]	0.902 ± 0.01 [a]	0.63 ± 0.31 [a]
F	4.63 ± 0.17 [c]	0.902 ± 0.00 [a]	0.64 ± 0.22 [a]
G	4.88 ± 0.27 [b]	0.905 ± 0.01 [a]	0.70 ± 0.32 [a]
H	4.68 ± 0.17 [c]	0.897 ± 0.00 [a]	0.74 ± 0.17 [a]
I	4.85 ± 0.23 [b]	0.909 ± 0.01 [a]	0.78 ± 0.36 [a]
Sig.	**	ns	ns

[a–c]: Different letters indicate statistical difference ($p < 0.05$) in each column. ** $p < 0.01$, ns: not significant. (Control: without starter culture, A: *L. sakei* S15, B: *L. plantarum* S91, C: *P. pentosaceus* 128b, D: *L. plantarum* S91 + *L. sakei* S15, E: *L. plantarum* S24 + *L. plantarum* S91 + *L. sakei* S15, F: *P. pentosaceus* S128b + *L. plantarum* S91 + *L. sakei* S15, G: *L. sakei* S15 + *S. carnosus* G109, H: *L. sakei* S15 + *L. plantarum* S91 + *S. carnosus* G109, I: *P. pentosaceus* S128b + *S. carnosus* G109 + *L. sakei* S15).

The use of ASC did not cause a significant difference in a_w values ($p > 0.05$) (Table 2). However, in a study it was revealed that a_w decreases more rapidly in sucuk produced with LAB than in naturally fermented sucuk [16]. These differences are probably related to the composition of the spontaneous microbiota [10].

TBARS is an indicator of lipid oxidation in meat products. It has been reported that the deterioration of sausages is caused not only by spoilage microorganisms but also by lipid oxidation. Lipid oxidation, in turn, affects product quality by producing off-odors and by promoting the formation of peroxides that are harmful to human health [42]. The TBARS values of sucuk produced with ASC are listed in Table 2. The use of ASC did not cause a significant difference in TBARS values ($p > 0.05$). On the other hand, in a study conducted on sucuk, the use of *L. sakei* S15 as a monoculture increased the TBARS value at the end of the ripening period, whereas certain mixed cultures resulted in lower TBARS values, which was attributed to the antioxidant activity of *S. carnosus* [19]. As presented in Table 2, the mean TBARS values obtained in this study were below 1 mg MDA/kg. TBARS values lower than 1 mg MDA/kg generally indicate minimal lipid oxidation, suggesting that the product is unlikely to develop rancid flavors. Values ranging from 1 to 2 mg MDA/kg reflect a moderate degree of lipid oxidation. In contrast, TBARS values exceeding 2 mg MDA/kg are indicative of advanced oxidative deterioration, accompanied

by noticeable flavor degradation and the development of off-flavors, particularly rancidity. It should be noted that TBARS levels can vary substantially depending on the specific processing conditions applied to the meat product. In fact, rancidity levels as high as 2–2.5 mg MDA/kg have been reported in certain processed meat products [43].

3.3. Color Values

The effects of the starter cultures on the color of the sucuk are shown in Figure 2. The results suggested that the use of starter cultures caused a statistical difference only in the L^* value among the color parameters examined ($p < 0.05$). The lowest L^* values were observed in the control group. However, the mean value of this group did not differ significantly from that of the group treated with only *L. plantarum* S91. These results indicate that starter culture inoculation had only a minor effect on the color characteristics of the products. Color formation and stability are important components of fermented sausage quality, and consumers often associate overall quality with color [44]. Kaban et al. [19] reported that sucuk samples containing ASC had higher L^* values than the control group ($p < 0.01$). In addition, in the same study, ASC showed no significant effect on the a^* value of the sucuk samples ($p > 0.05$). Van Ba et al. [45] reported lower L^* values in sausage samples inoculated with *S. carnosus* + *S. xylosus* + *D. hansenii* + *L. curvatus*, and higher L^* values in those inoculated with *S. xylosus* + *L. plantarum* ($p < 0.05$). On the other hand, Casaburi et al. [35] found no effect of starter inoculation or pH on color changes in fermented sausages.

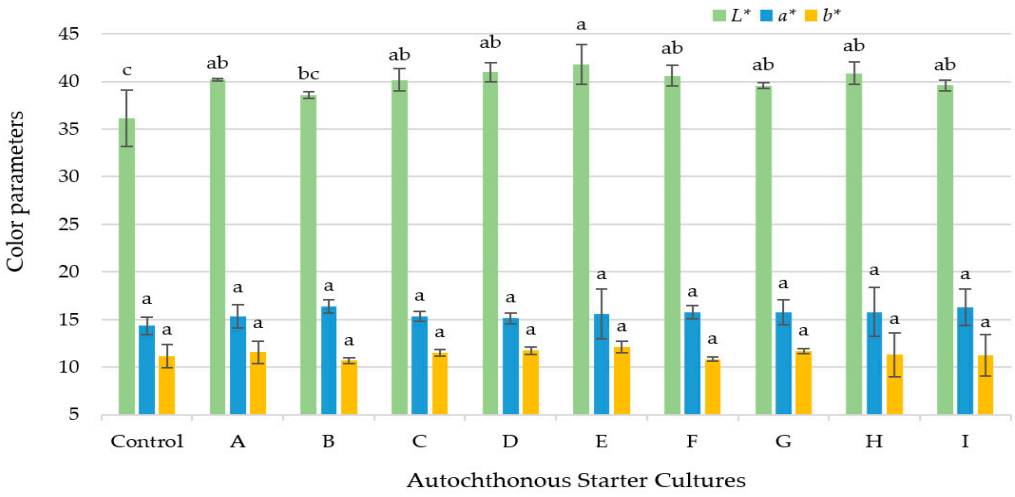


Figure 2. The effects of starter cultures on color values of sucuk samples (Control: without starter culture, A: *L. sakei* S15, B: *L. plantarum* S91, C: *P. pentosaceus* 128b, D: *L. plantarum* S91 + *L. sakei* S15, E: *L. plantarum* S24 + *L. plantarum* S91 + *L. sakei* S15, F: *P. pentosaceus* S128b + *L. plantarum* S91 + *L. sakei* S15, G: *L. sakei* S15 + *S. carnosus* G109, H: *L. sakei* S15 + *L. plantarum* S91 + *S. carnosus* G109, I: *P. pentosaceus* S128b + *S. carnosus* G109 + *L. sakei* S15). (a–c: Different lowercase letters indicate significant differences between autochthonous starter culture for each color parameter) ($p < 0.05$).

3.4. Volatile Compounds

The formation of volatile compounds has been attributed to lipid oxidation, protein hydrolysis, and amino acid catabolism. During the ripening process of sausages, a wide array of volatile compounds is generated as a result of these biochemical and enzymatic reactions [46]. In this study, 68 compounds belonging to 12 different chemical groups were identified, including aldehydes, alcohols, sulfur compounds, aliphatic hydrocarbons, esters, acids, ketones, aromatic hydrocarbons, terpenes, furans, phenols, and nitrogenous compounds (Table 3). Many non-volatile compounds, such as peptides and amino acids, are produced by the degradation of meat proteins during the ripening of fermented sausages [47]. However, the sources of volatile compounds vary, and these compounds may

also be generated through secondary reactions between substances produced by various catabolic processes [48]. It is known that flavor development in fermented sausages is influenced by spices in the formulation, process conditions, and the presence of starter cultures [15]. Proteolysis also plays an important role in the aroma of dry-fermented sausages. As a result of proteolytic reactions, the levels of volatile compound precursors, flavor-active free amino acids, and low-molecular-weight peptides (non-protein nitrogenous substances) increase [49–51]. Factors such as formulation, starter culture, and production conditions, which vary from product to product, are also known to influence proteolysis [52].

Aldehydes are typical products of lipid oxidation, with hexanal and nonanal being formed through lipid auto-oxidation and characterized by low threshold values. These compounds are considered major contributors to the flavor of processed meat products [53]. The use of ASC in sucuk samples showed no significant effect on hexanal and heptanal ($p > 0.05$) but had a significant effect on acetaldehyde, decanal, 3-phenyl-2-methylpropanal ($p < 0.01$), and octanal ($p < 0.05$) (Table 3). Changes in lipid content during ripening also significantly affect the sensory properties of the product [46,54]. Free fatty acids formed as a result of lipolysis are good substrates for autoxidation. The end products of autoxidation (aldehydes, alcohols, ketones, furans, etc.) are volatile compounds that play an important role in odor and flavor development. In these products, different factors such as product formulation, meat particle size, pH, salt, nitrite, spices, and antioxidants are known to affect lipid oxidation [46].

ASC treatment was significantly associated with benzeneethanol and 4-(1-methylethyl) benzenemethanol ($p < 0.01$). In this study, ethyl alcohol levels were significantly higher in the control group than in the other groups ($p < 0.05$) (Table 3). Alcohols are primarily derived from the reduction of methyl ketones, carbohydrate metabolism, amino acid catabolism, and lipid oxidation, and therefore play an important role in determining the flavor profiles of fermented sausages [22].

Sulfur compounds, with their low threshold values, can significantly affect the sensory properties of food, even in small amounts. These compounds may originate from garlic or may result from amino acid catabolism. The characteristic aroma of garlic is mainly attributed to diallyl disulfide and diallyl thiosulfinate [55]. In the present study, diallyl disulfide, methyl-2-propenyl disulfide, 3,3′-thiobis-1-propene ($p < 0.01$), allyl mercaptan, and di-2-propenyl disulfide ($p < 0.05$) were significantly affected by the addition of ASC (Table 3). In terms of 3,3′-thiobis-1-propene, the control group and groups A, G, and I, which did not contain *L. plantarum*, showed similar results (Table 3).

Ketones are characteristic flavor compounds in fermented sausages that contribute to floral, creamy, fatty, and fruity notes [56]. In this study, ASC inoculation in sucuk samples had a significant effect on 3-hydroxy-2-butanone ($p < 0.01$) and 2-nonanone ($p < 0.05$).

Fermentation and storage of meat lead to the production of various compounds, including acetic acid, propionic acid, butyric acid, acetaldehyde, diacetyl, and acetoin, as a result of lactic acid metabolism [4]. Acetic acid, a product of carbohydrate metabolism, can also be formed via lipid or amino acid catabolism [49,57]. As can be seen from Table 3, acetic acid and propionic acid levels in sucuk samples were significantly affected by ASC inoculation ($p < 0.01$). Groups D, E, F, H, and I had higher acetic acid abundance than the other groups, including the control group. In groups B, D, F and H, high abundances were observed in terms of propionic acid (Table 3).

Table 3. The effects of autochthonous strains on volatile compounds (AUx10^6) of sucuk (mean± standard deviation).

Identified Compounds	KI	RI	Control	A	B	C	D	E	F	G	H	I	Sig.
Acetaldehyde	<500	a	6.74 ± 1.52 ^{d}	19.38 ± 14.93 ^{bcd}	32.68 ± 3.69 ^{abc}	20.72 ± 11.32 ^{abcd}	38.07 ± 3.45 ^{a}	35.93 ± 3.30 ^{ab}	27.73 ± 16.11 ^{abc}	16.08 ± 10.57 ^{cd}	37.81 ± 4.79 ^{a}	25.49 ± 21.01 ^{abc}	**
Ethanol	512	a	34.84 ± 20.50 ^{a}	14.64 ± 9.83 ^{b}	4.16 ± 5.47 ^{bc}	13.12 ± 8.82 ^{b}	5.4 ± 3.47 ^{bc}	5.11 ± 2.49 ^{bc}	4.59 ± 2.01 ^{bc}	1.68 ± 1.52 ^{c}	6.62 ± 4.21 ^{bc}	5.15 ± 3.41 ^{bc}	**
Carbon disulfide	536	c	0.00 ± 0.00	1.87 ± 2.86	6.60 ± 6.96	0.54 ± 0.63	1.01 ± 1.98	4.54 ± 2.76	1.27 ± 2.01	1.39 ± 2.08	2.19 ± 1.67	1.11 ± 1.77	ns
Allyl mercaptan	574	b	30.21 ± 10.14 ^{c}	86.21 ± 28.33 ^{abc}	111.06 ± 35.9 ^{ab}	59.75 ± 32.72 ^{bc}	117.43 ± 67.38 ^{ab}	111.69 ± 38.06 ^{ab}	122.46 ± 58.4 ^{ab}	78.32 ± 24.70 ^{abc}	144.84 ± 51.17 ^{a}	61.57 ± 23.70 ^{bc}	*
Hexane	600	a	145.38 ± 97.23	172.84 ± 105.37	115.63 ± 32.5	164.95 ± 95.69	214.74 ± 142.76	114.96 ± 59.50	137.3 ± 74.05	110.25 ± 91.61	147.61 ± 82.99	119.29 ± 71.64	ns
Ethyl acetate	639	a	1.33 ± 1.02	1.30 ± 1.53	2.53 ± 0.28	2.26 ± 1.89	2.06 ± 1.47	1.16 ± 1.38	1.53 ± 1.11	1.45 ± 1.02	2.42 ± 0.90	2.74 ± 1.59	ns
Acetic acid	710	a	7.95 ± 3.39 ^{e}	18.44 ± 5.24 ^{de}	35.51 ± 11.18 ^{bc}	19.54 ± 3.56 ^{de}	45.85 ± 13.20 ^{ab}	56.00 ± 15.03 ^{a}	50.94 ± 12.01 ^{ab}	30.34 ± 11.24 ^{cd}	53.25 ± 2.93 ^{a}	48.69 ± 11.40 ^{ab}	**
Allyl methyl sulfide	730	bc	6.17 ± 2.76	6.83 ± 3.55	7.24 ± 3.94	8.2 ± 5.89	5.63 ± 4.68	4.46 ± 1.72	6.42 ± 5.31	7.82 ± 4.66	6.02 ± 3.90	7.13 ± 4.03	ns
3-hydroxy-2-butanone	779	a	0.65 ± 0.76 ^{c}	0.54 ± 0.62 ^{c}	7.05 ± 2.72 ^{a}	3.29 ± 2.09 ^{bc}	4.06 ± 2.03 ^{b}	5.89 ± 2.79 ^{ab}	4.4 ± 2.60 ^{ab}	0.62 ± 0.42 ^{c}	5.79 ± 1.17 ^{ab}	0.75 ± 0.64 ^{c}	**
1-methyl-1H-pyrrole	786	b	0.00 ± 00 ^{d}	0.40 ± 0.46 ^{bcd}	1.13 ± 0.82 ^{ab}	0.85 ± 0.69 ^{abc}	1.09 ± 0.45 ^{b}	1.04 ± 0.38 ^{ab}	1.26 ± 0.60 ^{a}	0.00 ± 00 ^{d}	1.35 ± 0.57 ^{a}	0.22 ± 0.45 ^{cd}	**
Toluene	798	a	0.40 ± 0.47	0.36 ± 0.41	1.68 ± 1.88	0.68 ± 0.88	0.64 ± 0.43	0.62 ± 0.73	0.97 ± 0.87	0.90 ± 0.80	0.95 ± 0.74	2.39 ± 2.49	ns
Hexanal	849	a	1.95 ± 2.41	2.09 ± 0.93	1.96 ± 1.90	1.93 ± 1.62	0.43 ± 0.26	2.27 ± 1.62	1.03 ± 0.42	0.80 ± 0.18	1.49 ± 1.13	1.87 ± 1.51	ns
Butanoic acid	878	b	0.43 ± 0.51	1.09 ± 0.50	1.44 ± 0.46	1.00 ± 0.47	1.32 ± 0.71	1.40 ± 0.76	1.60 ± 0.83	1.38 ± 0.76	1.74 ± 0.71	1.55 ± 0.41	ns
3,3′-thiobis-1-propene	888	b	15.55 ± 4.13 ^{a}	13.14 ± 4.51 ^{ab}	4.86 ± 1.62 ^{c}	7.4 ± 3.06 ^{bc}	4.94 ± 2.06 ^{c}	4.38 ± 2.61 ^{c}	5.44 ± 2.61 ^{c}	18.87 ± 7.39 ^{a}	6.51 ± 3.38 ^{bc}	14.86 ± 9.70 ^{a}	**
Propionic acid	891	b	0.38 ± 0.50 ^{c}	2.34 ± 0.91 ^{c}	15.96 ± 5.41 ^{a}	0.79 ± 0.69 ^{c}	10.79 ± 7.03 ^{ab}	7.1 ± 5.79 ^{bc}	11.79 ± 5.68 ^{ab}	2.69 ± 1.11 ^{c}	13.31 ± 8.15 ^{ab}	2.14 ± 1.13 ^{c}	**
1-hexanol	930	a	0.61 ± 0.59	0.27 ± 0.32	0.44 ± 0.51	0.64 ± 0.78	0.54 ± 0.64	0.38 ± 0.76	0.78 ± 0.91	0.00 ± 0.00	0.28 ± 0.48	0.44 ± 0.54	ns
Butyl propionate	939	b	0.40 ± 0.35	0.49 ± 0.19	0.97 ± 0.76	0.74 ± 1.48	0.38 ± 0.45	0.51 ± 0.62	0.98 ± 0.77	0.75 ± 0.37	0.81 ± 0.62	0.40 ± 0.47	ns
α-thujen	944	b	0.66 ± 0.30 ^{bc}	0.62 ± 0.27 ^{bc}	1.37 ± 0.27 ^{a}	0.8 ± 0.29 ^{bc}	1.01 ± 0.38 ^{b}	0.79 ± 0.17 ^{bc}	0.95 ± 0.37 ^{bc}	0.65 ± 0.33 ^{bc}	0.94 ± 0.41 ^{bc}	0.60 ± 0.33 ^{c}	**
Heptanal	955	a	0.00 ± 0.00	0.00 ± 0.00	0.43 ± 0.29	0.19 ± 0.38	0.17 ± 0.34	0.15 ± 0.30	0.16 ± 0.32	0.15 ± 0.31	0.35 ± 0.40	0.29 ± 0.33	ns
α-pinene	950	c	4.74 ± 3.18 ^{b}	6.93 ± 0.61 ^{a}	7.97 ± 0.90 ^{a}	7.11 ± 0.69 ^{a}	8.80 ± 1.01 ^{a}	8.03 ± 0.50 ^{a}	7.94 ± 0.83 ^{a}	7.31 ± 0.52 ^{a}	8.46 ± 0.89 ^{a}	7.04 ± 0.83 ^{a}	**
Methyl-2-propenyl disulfide	954	b	12.48 ± 4.47 ^{ab}	9.33 ± 3.71 ^{b}	2.59 ± 2.21 ^{c}	8.86 ± 4.76 ^{b}	1.33 ± 0.84 ^{c}	0.96 ± 0.76 ^{c}	1.28 ± 0.65 ^{c}	14.68 ± 5.24 ^{a}	1.80 ± 1.09 ^{c}	10.83 ± 5.48 ^{ab}	**
Camphene	970	c	0.29 ± 0.19	0.41 ± 0.04	0.36 ± 0.19	0.41 ± 0.10	0.51 ± 0.08	0.45 ± 0.03	0.49 ± 0.08	0.29 ± 0.20	0.40 ± 0.27	0.24 ± 0.28	ns
β-pinene	996	b	12.01 ± 1.34	9.84 ± 6.61	10.14 ± 6.84	13.77 ± 2.52	14.63 ± 2.34	8.03 ± 9.28	20.92 ± 22.81	19.38 ± 5.47	18.19 ± 3.24	15.59 ± 1.23	ns
β-myrcene	998	b	16.67 ± 4.89	19.50 ± 17.93	41.22 ± 4.05	23.48 ± 11.9	35.43 ± 15.22	36.52 ± 8.87	25.70 ± 23.31	9.66 ± 11.77	34.98 ± 15.25	20.81 ± 10.08	ns
2-pentyl-furan	1021	b	0.11 ± 0.21	0.29 ± 0.33	0.46 ± 0.17	0.39 ± 0.33	0.27 ± 0.34	1.03 ± 0.64	0.50 ± 0.14	1.14 ± 0.66	1.35 ± 1.57	1.21 ± 0.64	ns
α-phellandrene	1024	b	7.11 ± 1.65 ^{c}	7.94 ± 2.51 ^{bc}	13.60 ± 2.72 ^{ab}	10.09 ± 4.47 ^{abc}	15.41 ± 5.48 ^{a}	13.41 ± 3.12 ^{ab}	15.00 ± 5.06 ^{a}	8.84 ± 2.61 ^{bc}	15.56 ± 4.27 ^{a}	9.23 ± 2.53 ^{bc}	**
3-carene	1026	b	39.48 ± 3.96 ^{e}	41.95 ± 5.34 ^{de}	51.73 ± 1.78 ^{bc}	46.54 ± 7.56 ^{cd}	60.93 ± 6.97 ^{a}	56.69 ± 4.21 ^{ab}	56.48 ± 6.34 ^{ab}	47.79 ± 3.78 ^{cd}	59.63 ± 5.13 ^{a}	47.07 ± 5.33 ^{cd}	**
6-methyl-5-hepten-2-one	1048	b,c	0.18 ± 0.21	0.18 ± 0.21	0.33 ± 0.28	0.31 ± 0.38	0.38 ± 0.31	0.43 ± 0.34	0.30 ± 0.35	0.20 ± 0.23	0.13 ± 0.26	0.23 ± 0.27	ns
α-terpinene	1049	b	1.79 ± 0.29 ^{d}	1.91 ± 0.51 ^{cd}	2.53 ± 1.28 ^{abcd}	2.42 ± 0.78 ^{bcd}	3.88 ± 1.18 ^{a}	3.27 ± 0.73 ^{abc}	3.65 ± 1.15 ^{ab}	2.06 ± 0.70 ^{cd}	3.57 ± 1.09 ^{ab}	1.99 ± 0.79 ^{cd}	**
Octanal	1051	a	0.78 ± 0.21 ^{bc}	0.43 ± 0.51 ^{c}	1.56 ± 0.19 ^{ab}	1.09 ± 0.52 ^{abc}	1.52 ± 0.62 ^{ab}	1.11 ± 0.84 ^{abc}	0.99 ± 0.77 ^{abc}	0.63 ± 0.44 ^{c}	1.78 ± 0.54 ^{a}	0.92 ± 0.34 ^{abc}	*

Table 3. *Cont.*

Identified Compounds	KI	RI	Control	A	B	C	D	E	F	G	H	I	Sig.
D-limonene	1054	b	58.72 ± 13.34 ^{b}	60.71 ± 17.37 ^{b}	91.14 ± 48.40 ^{ab}	72.84 ± 28.04 ^{ab}	108.18 ± 31.58 ^{a}	92.09 ± 14.64 ^{ab}	102.97 ± 28.8 ^{a}	66.74 ± 20.43 ^{ab}	104.22 ± 30.90 ^{a}	67.02 ± 21.68 ^{ab}	*
β-phellandrene	1060	b	0.73 ± 0.36 ^{b}	0.84 ± 0.44 ^{b}	2.62 ± 1.43 ^{a}	0.96 ± 0.66 ^{b}	1.14 ± 0.47 ^{b}	1.28 ± 0.55 ^{b}	1.28 ± 0.67 ^{b}	1.19 ± 0.46 ^{b}	1.55 ± 0.70 ^{b}	1.27 ± 0.58 ^{b}	*
1-methyl-2-(1-methylethyl)-benzene	1072	c	65.32 ± 10.16	65.6 ± 13.97	87.8 ± 15.47	67.95 ± 20.45	103.19 ± 32.9	86.81 ± 27	99.21 ± 30.45	68.64 ± 21.53	99.41 ± 40.03	68.2 ± 29.09	ns
Eucalyptol	1075	c	1.73 ± 0.18	1.88 ± 0.50	2.43 ± 1.30	2.17 ± 0.52	1.99 ± 1.21	2.17 ± 0.85	2.18 ± 1.44	1.67 ± 0.45	2.43 ± 0.95	2.21 ± 0.5	ns
γ-terpinene	1089	c	38.89 ± 10.21	43.51 ± 14.32	58.47 ± 24.96	54 ± 20.51	81.28 ± 28.70	68.82 ± 23.28	78.78 ± 27.77	52.14 ± 15.34	81.25 ± 21.92	53.93 ± 10.62	ns
Undecane	1100	a	0.52 ± 0.23	0.55 ± 0.42	0.57 ± 0.09	0.71 ± 0.37	0.62 ± 0.44	0.58 ± 0.19	0.78 ± 0.24	0.44 ± 0.25	0.59 ± 0.18	0.27 ± 0.18	ns
2-carene	1120	c	0.77 ± 0.61	0.83 ± 0.70	1.05 ± 0.80	1.17 ± 0.48	0.83 ± 1.10	0.93 ± 1.08	1.36 ± 0.92	0.17 ± 0.34	0.95 ± 1.13	0.42 ± 0.51	ns
Terpinolene	1131	b	1.99 ± 0.51	2.32 ± 1.15	3.8 ± 0.78	2.85 ± 1.23	4.07 ± 1.78	3.32 ± 0.85	4.10 ± 1.50	2.62 ± 1.01	4.01 ± 1.43	2.62 ± 0.88	ns
Propyl hexanoate	1134	b	2.16 ± 0.26	0.95 ± 0.21	2.86 ± 0.54	2.41 ± 3.65	1.08 ± 1.03	1.49 ± 1.78	1.97 ± 2.40	0.76 ± 0.99	2.21 ± 1.79	0.33 ± 0.24	ns
Benzyl alcohol	1135	c	0.73 ± 0.36	1.3 ± 0.91	0.85 ± 0.77	1.54 ± 0.22	1.59 ± 0.70	0.7 ± 0.82	0.95 ± 0.79	0.99 ± 1.25	0.74 ± 0.88	0.00 ± 0.00	ns
1-methyl-4-(1-methylethenyl)-benzene	1136	c	3.36 ± 0.42 ^{bc}	3.67 ± 0.44 ^{bc}	4.79 ± 2.52 ^{abc}	2.91 ± 0.44 ^{c}	4.77 ± 0.81 ^{abc}	6.27 ± 0.85 ^{a}	6.09 ± 1.90 ^{a}	4.72 ± 0.65 ^{abc}	6.73 ± 1.82 ^{a}	5.42 ± 0.89 ^{ab}	**
Diallyl disulfide	1038	a	68.56 ± 24.79 ^{ab}	50.93 ± 24.11 ^{abc}	13.75 ± 4.95 ^{d}	43.81 ± 22.92 ^{bcd}	20.29 ± 10.72 ^{cd}	16.71 ± 13.25 ^{cd}	21.46 ± 9.75 ^{cd}	83.74 ± 38.10 ^{a}	25.96 ± 13.52 ^{cd}	68.34 ± 34.93 ^{ab}	**
2-nonanone	1140	c	0.00 ± 0.00 ^{c}	2.62 ± 2.05 ^{a}	0.43 ± 0.50 ^{bc}	0.00 ± 0.00 ^{c}	0.62 ± 0.54 ^{bc}	0.65 ± 0.76 ^{bc}	0.83 ± 0.97 ^{bc}	0.49 ± 0.97 ^{bc}	1.60 ± 1.02 ^{ab}	0.57 ± 0.67 ^{bc}	*
Linalool	1161	a	3.67 ± 0.35 ^{c}	3.62 ± 0.75 ^{c}	3.79 ± 1.89 ^{c}	4.22 ± 1.09 ^{bc}	5.70 ± 1.33 ^{ab}	5.18 ± 0.89 ^{abc}	5.64 ± 1.25 ^{abc}	4.4 ± 1.01 ^{bc}	6.22 ± 1.26 ^{a}	4.51 ± 0.74 ^{bc}	*
di-2-propenyl disulfide	1168	c	7.61 ± 2.15 ^{abcd}	7.72 ± 1.99 ^{abcd}	3.25 ± 2.23 ^{d}	8.19 ± 4.49 ^{bc}	5.89 ± 2.48 ^{cd}	5.13 ± 2.32 ^{cd}	6.30 ± 2.19 ^{bcd}	10.73 ± 3.40 ^{ab}	8.15 ± 3.09 ^{bc}	11.58 ± 4.03 ^{a}	*
6-methyl-3,5-heptadiene-2-one	1175	c	0.51 ± 0.38 ^{bc}	0.55 ± 0.39 ^{bc}	1.09 ± 0.83 ^{abc}	0.22 ± 0.30 ^{c}	1.36 ± 0.38 ^{ab}	0.89 ± 0.60 ^{abc}	0.35 ± 0.70 ^{c}	0.35 ± 0.43 ^{c}	1.58 ± 1.10 ^{a}	0.90 ± 0.15 ^{abc}	*
4-methyl-phenol	1184	c	0.17 ± 0.34 ^{bc}	0.28 ± 0.33 ^{abc}	0.00 ± 0.00 ^{c}	0.14 ± 0.27 ^{bc}	0.49 ± 0.34 ^{ab}	0.39 ± 0.53 ^{abc}	0.00 ± 0.00 ^{c}	0.00 ± 0.00 ^{c}	0.67 ± 0.25 ^{a}	0.00 ± 0.00 ^{c}	*
Dodecane	1200	c	0.69 ± 0.30	0.32 ± 0.37	0.52 ± 0.39	0.82 ± 0.65	0.98 ± 0.47	0.76 ± 0.56	0.53 ± 0.36	0.66 ± 0.46	1.06 ± 0.15	0.98 ± 0.27	ns
Benzeneethanol	1214	c	0.00 ± 0.00 ^{c}	0.00 ± 0.00 ^{c}	00.00 ± 0.00 ^{c}	0.21 ± 0.42 ^{bc}	0.00 ± 0.00 ^{c}	0.00 ± 0.00 ^{c}	0.32 ± 0.64 ^{bc}	0.92 ± 0.30 ^{a}	0.65 ± 0.46 ^{ab}	0.79 ± 0.17 ^{a}	**
Hexyl butanoate	1221	b	4.30 ± 1.50	1.61 ± 0.60	2.68 ± 1.43	4.94 ± 3.52	2.72 ± 0.43	3.12 ± 1.63	3.56 ± 1.46	2.27 ± 0.68	3.98 ± 1.55	2.17 ± 0.46	ns
p-Menth-2-ene-1,8-diol	1228	c	0.39 ± 0.33	0.38 ± 0.09	0.47 ± 0.10	0.37 ± 0.27	0.12 ± 0.23	0.19 ± 0.21	0.60 ± 0.11	0.18 ± 0.35	0.58 ± 0.39	0.36 ± 0.41	ns
Terpinene-4-ol	1233	b,c	2.16 ± 0.24 ^{cd}	1.50 ± 1.00 ^{d}	2.63 ± 1.67 ^{bcd}	2.26 ± 0.54 ^{cd}	2.99 ± 0.47 ^{bc}	3.25 ± 0.18 ^{abc}	3.79 ± 0.68 ^{ab}	2.42 ± 0.44 ^{cd}	4.18 ± 0.38 ^{a}	2.64 ± 0.31 ^{bcd}	**
Phenol	1249	c	0.34 ± 0.41	0.17 ± 0.34	0.34 ± 0.28	0.24 ± 0.30	0.00 ± 0.00	0.15 ± 0.30	0.24 ± 0.47	0.20 ± 0.26	0.13 ± 0.26	0.14 ± 0.17	ns
Decanal	1258	a	1.00 ± 1.02 ^{bc}	0.44 ± 0.69 ^{c}	2.14 ± 0.15 ^{a}	1.55 ± 0.54 ^{ab}	0.26 ± 0.31 ^{c}	0.33 ± 0.65 ^{c}	0.49 ± 0.73 ^{c}	0.41 ± 0.09 ^{c}	0.31 ± 0.61 ^{c}	1.85 ± 1.24 ^{ab}	**
α-terpineol	1264	b,c	2.11 ± 0.13 ^{cde}	0.95 ± 1.09 ^{e}	2.85 ± 1.60 ^{abcd}	1.95 ± 0.46 ^{de}	3.23 ± 0.54 ^{abc}	3.56 ± 0.40 ^{ab}	3.47 ± 0.45 ^{ab}	1.76 ± 1.23 ^{e}	3.88 ± 0.66 ^{a}	2.43 ± 0.23 ^{bcd}	**
Benzoic acid 2-hydroxy-methyl ester	1273	c	0.37 ± 0.43 ^{bc}	0.64 ± 0.10 ^{abc}	1.08 ± 0.40 ^{a}	0.21 ± 0.43 ^{c}	0.25 ± 0.29 ^{c}	0.31 ± 0.38 ^{bc}	0.62 ± 0.13 ^{abc}	0.6 ± 0.45 ^{abc}	0.82 ± 0.11 ^{ab}	0.93 ± 0.11 ^{a}	**
3-phenyl-2-methyl-propanal	1332	b,c	340.98 ± 94.79 ^{bc}	409.94 ± 45.27 ^{ab}	83.13 ± 35.79 ^{d}	311.77 ± 124.86 ^{c}	83.17 ± 10.07 ^{d}	63.6 ± 33.26 ^{d}	83.06 ± 8.22 ^{d}	437.41 ± 63.67 ^{a}	124.35 ± 22.1 ^{d}	462.28 ± 73.41 ^{a}	**
Camphene	1362	c	5.37 ± 0.36 ^{b}	5.35 ± 0.47 ^{b}	14.59 ± 10.28 ^{a}	4.33 ± 1.98 ^{b}	5.48 ± 1.75 ^{b}	7.17 ± 3.22 ^{b}	7.55 ± 2.08 ^{b}	4.64 ± 1.31 ^{b}	5.10 ± 5.93 ^{b}	4.38 ± 0.45 ^{b}	*

Table 3. *Cont.*

Identified Compounds	KI	RI	Control	A	B	C	D	E	F	G	H	I	Sig.
3-carvomenthenone	1364	c	1.54 ± 1.07	1.15 ± 0.49	2.33 ± 2.87	0.58 ± 0.44	1.2 ± 0.95	1.62 ± 3.25	2.4 ± 2.85	2.08 ± 1.83	3.38 ± 4.01	2.83 ± 0.35	ns
Phellandral	1368	c	2.99 ± 2.04 ^{abcd}	3.64 ± 0.56 ^{abc}	1.73 ± 1.43 ^{d}	2.47 ± 1.06 ^{bcd}	1.97 ± 0.46 ^{cd}	2.97 ± 1.42 ^{abcd}	2.9 ± 0.68 ^{abcd}	3.71 ± 0.55 ^{abc}	4.15 ± 1.04 ^{ab}	4.48 ± 0.52 ^{a}	*
δ-elemene	1371	c	2.65 ± 0.78	2.88 ± 2.56	2.81 ± 2.42	2.83 ± 1.30	4.15 ± 0.40	5.16 ± 1.01	5.12 ± 1.98	4.58 ± 2.22	5.28 ± 0.84	3.86 ± 0.45	ns
α-hydroxyphenylacetic acid	1376	c	2.38 ± 0.81	3.27 ± 2.86	0.66 ± 0.68	2.49 ± 1.15	1.30 ± 0.91	1.00 ± 1.29	1.23 ± 0.82	5.30 ± 6.04	2.51 ± 0.76	4.46 ± 0.56	ns
4-(1-methylethyl) benzenemethanol	1380	b	76.73 ± 24.88 ^{bc}	101.73 ± 15.98 ^{ab}	56.92 ± 28.91 ^{c}	73.05 ± 29.62 ^{bc}	60.28 ± 14.06 ^{c}	69.13 ± 24.02 ^{bc}	69.98 ± 10.95 ^{bc}	92.40 ± 41.13 ^{bc}	90.30 ± 20.85 ^{bc}	129.31 ± 13.77 ^{a}	**
3-methyl-4-isopropylphenol	1392	c	17.19 ± 5.94	22.93 ± 3.09	19.00 ± 10.19	16.58 ± 7.26	18.85 ± 3.50	22.80± 5.02	22.46 ± 1.75	20.36 ± 12.25	28.82 ± 5.26	31.52 ± 2.09	ns
Copaene	1432	b	4.92 ± 0.86	6.93 ± 1.66	4.32 ± 4.67	5.17 ± 1.94	6.00 ± 0.97	7.80 ± 1.14	8.09 ± 2.39	7.81 ± 1.96	8.20 ± 1.41	7.95 ± 0.44	ns
β-elemene	1438	c	0.81 ± 1.17	0.36 ± 0.72	2.08 ± 3.27	0.29 ± 0.34	0.36 ± 0.42	1.17 ± 1.44	0.26 ± 0.52	0.27 ± 0.53	0.90 ± 1.04	0.95 ± 1.10	ns
Caryophyllene	1489	b,c	21.15 ± 4.28 ^{c}	25.92 ± 4.00 ^{bc}	24.92 ± 12.31 ^{bc}	28.22 ± 12.93 ^{bc}	33.48 ± 4.11 ^{ab}	34.75 ± 7.89 ^{ab}	35.66 ± 7.34 ^{ab}	30.52 ± 3.67 ^{abc}	40.73 ± 5.53 ^{a}	33.36 ± 1.06 ^{ab}	*
α-caryophyllene	1492	c	1.48 ± 0.48	1.89 ± 0.50	1.29 ± 0.76	1.62 ± 0.90	2.04 ± 0.25	2.10 ± 0.73	2.44 ± 0.73	6.47 ± 7.96	2.39 ± 0.39	2.54 ± 0.15	ns

$^{a–e}$: Different letters indicate statistical difference ($p < 0.05$) in each line. * $p < 0.05$, ** $p < 0.01$, ns: Not significant. Results are expressed in Arbitrary Units ($\times 10^6$). KI: Kovats index calculated for DB-624 capillary column (J&W Scientific. 60 m × 0.25 mm i.d. × 1.4 µm film) installed on a GC/MS. 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. (Control: without starter culture, A: *L. sakei* S15, B: *L. plantarum* S91, C: *P. pentosaceus* 128b, D: *L. plantarum* S91 + *L. sakei* S15, E: *L. plantarum* S24 + *L. plantarum* S91 + *L. sakei* S15, F: *P. pentosaceus* S128b + *L. plantarum* S91 + *L. sakei* S15, G: *L. sakei* S15 + *S. carnosus* G109, H: *L. sakei* S15 + *L. plantarum* S91 + *S. carnosus* G109, I: *P. pentosaceus* S128b + *S. carnosus* G109 + *L. sakei* S15).

Esters in meat products are typically generated through the esterification of carboxylic acids and alcohols. Moreover, lactic acid bacteria and staphylococci are known to exhibit esterification activity as well [58]. In the present study, ethyl acetate, butyl propionate, propyl hexanoate, hexyl butanoate and benzoic acid 2-hydroxy-methyl ester were determined in the samples. Benzoic acid 2-hydroxy-methyl ester was only significantly affected by ASC addition ($p < 0.01$), and the groups B and I showed high abundance. However, the mean abundances of these groups did not differ from those of F, G, and H (Table 3).

1-methyl-1H-pyrrole, which is among the nitrogenous compounds, was affected by the use of ASC. The highest abundance was determined in groups F and H, which contain both *L. sakei* S15 and *L. plantarum* S91. However, no statistical difference was determined between these groups and groups B, C and E. Indeed, it is stated that microbial degradation of amino acids may be effective in the formation of compounds such as straight-chain sulfur compounds, pyrroles, pyrazines, and thiols [59].

In the present study, ASC use had no significant effect on the aliphatic hydrocarbon and furan compounds ($p > 0.05$) (Table 3). On the other hand, among aromatic hydrocarbons, 1-methyl-4-(1-methylethenyl)-benzene was affected by ASC at $p < 0.01$ level. On the other hand, of the three phenol compounds identified, ASC use showed a significant effect only on 4-methyl-phenol at the $p < 0.05$ level (Table 3), and the highest abundance was found in the groups E, F and H, which contain both *L. sakei* S15 and *L. plantarum* S91. However, no statistical difference was found between the mean values of these groups and the mean values of groups B, D, G and I (Table 3).

Terpenes primarily originate from spices used in the production of fermented sausages, as well as from the meat itself, which is influenced by animal nutrition [18]. In the present study, the use of ASC significantly affected α-thujene, α-pinene, α-phellandrene, 3-carene, terpinene-4-ol, α-terpinene and α-terpineol ($p < 0.01$), as well as β-phellandrene, D-limonene, linalool, camphene, phellandral, and caryophyllene ($p < 0.05$). Kaban and Kaya [16] and Kargozari et al. [9] also reported that ASCs influence many terpene compounds in sucuk. The highest mean abundances of α-thujene, β-phellandrene, and camphene were found in group B (*L. plantarum* S91), and this group was statistically different from the other groups. However, the lowest mean abundance of α-pinene were observed in the control group. The highest abundances of α-phellandrene, 3-carene, D-limonene, α-terpinene, linalool, and terpinene-4-ol were determined in groups containing both *L. sakei* S15 and *L. plantarum* S91. The lowest mean abundances of α-terpineol were found in groups A, C, and G. On the other hand, while the highest abundances of phellandral were found in group I, the use of mixed culture increased the caryophyllene content (Table 3). Terpenes are compounds that play a significant role in the volatile profile of sucuk [7]. These compounds may originate from spices, but they also have the potential to migrate into meat from animal feed. Additionally, studies on sucuk were determined that starter cultures affect terpene compounds [16,19,60], suggesting that this result may be due to terpene biotransformation by microorganisms. This study also determined that different ASC exhibited varying effects on many terpene compounds.

Figure 3 presents a correlation heat map illustrating the relationships between the starter cultures and volatile compounds. The analysis revealed differences among the starter cultures, with the use of different cultures resulting in the formation of two main distinct clusters. The use of different starter cultures resulted in significant differences in the volatile compound profiles. However, the most significant result is that all groups containing *L. plantarum* (B, D, E, F, and H) were clustered together in one cluster, indicating that *L. plantarum*, when used alone or with other microorganisms, produces a similar volatile profile (Figure 3).

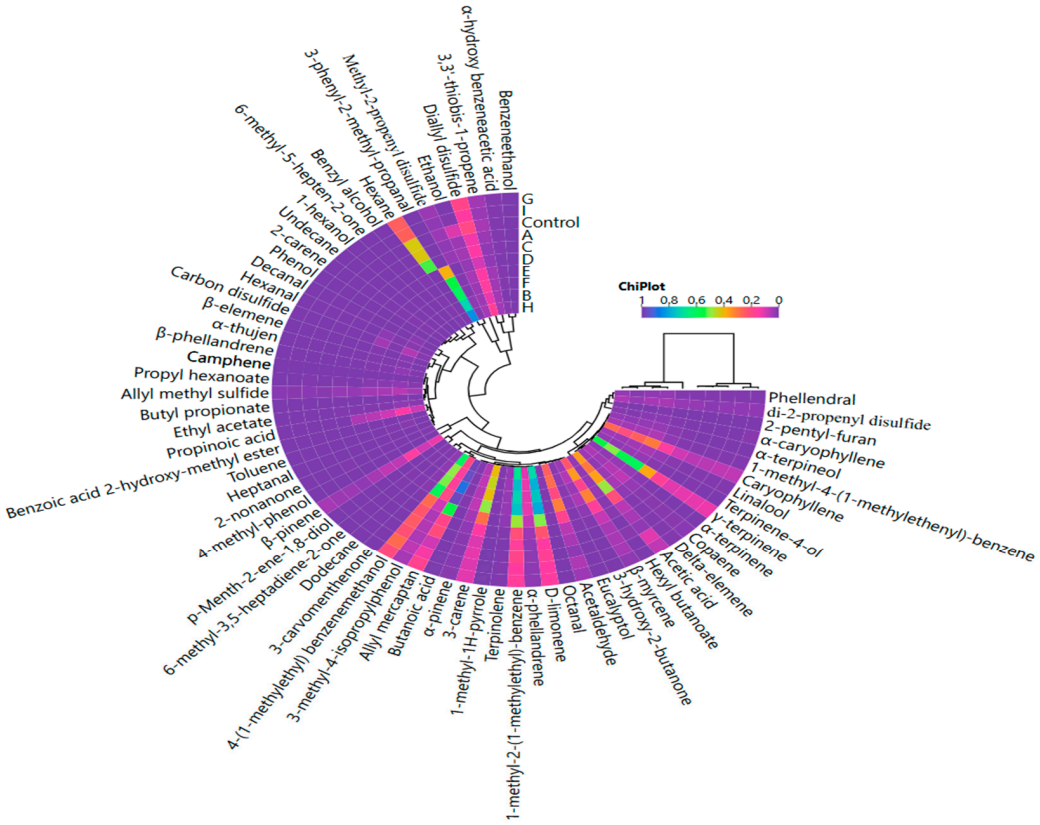


Figure 3. Cluster analysis of the correlation heat map illustrating the relationships between starter cultures and volatile compounds(Control: without starter culture, A: *L. sakei* S15, B: *L. plantarum* S91, C: *P. pentosaceus* 128b, D: *L. plantarum* S91 + *L. sakei* S15, E: *L. plantarum* S24 + *L. plantarum* S91 + *L. sakei* S15, F: *P. pentosaceus* S128b + *L. plantarum* S91 + *L. sakei* S15, G: *L. sakei* S15 + *S. carnosus* G109, H: *L. sakei* S15 + *L. plantarum* S91 + *S. carnosus* G109, I: *P. pentosaceus* S128b + *S. carnosus* G109 + *L. sakei* S15).

Principal component analysis (PCA) was conducted to assess the relationships among starter cultures and volatile compounds (Figure 4). The PC-1 explained 49.20% of the variation, and PC-2 explained 26.40% of the variation. The total variance was fully explained (75.60%) by the first two principal components. All groups containing *L. plantarum* (B, D, E, F and H) were located on the positive side of PC-1 and were separated from the other groups (control, A, C, G and I). Furthermore, phenols, furans, acids, terpenes, aromatic hydrocarbons, ketones, nitrogenous compounds, esters, and aliphatic hydrocarbons were located on the positive side of PC-1 and showed positive correlations with all groups containing *L. plantarum*. The groups without *L. plantarum* and the control group were on the negative side of PC1 and exhibited positive correlations with alcohols, aldehydes and sulfide compounds (Figure 4). In contrast, alcohols and aldehydes formed as a result of lipid oxidation showed negative correlations with groups containing *L. plantarum*. This result is thought to be due to the antioxidant properties of *L. plantarum* strains. Indeed, Mei et al. [61] reported that *L. plantarum* strains with antioxidant properties are effective in inhibiting lipid and protein oxidation and contribute to a better flavor in the sausage.

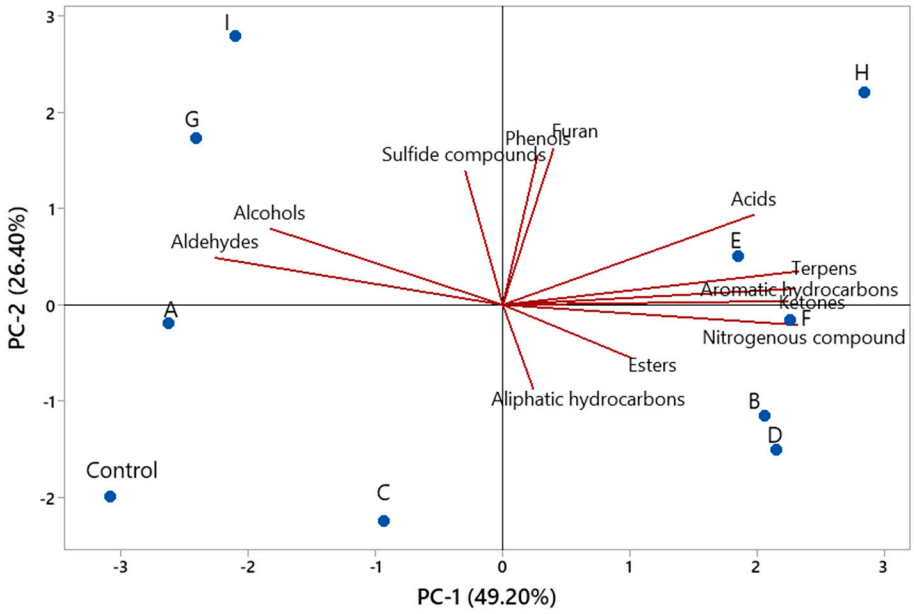


Figure 4. PCA biplot of the relationships between starter cultures and volatile compounds (Control: without starter culture, A: *L. sakei* S15, B: *L. plantarum* S91, C: *P. pentosaceus* 128b, D: *L. plantarum* S91 + *L. sakei* S15, E: *L. plantarum* S24 + *L. plantarum* S91 + *L. sakei* S15, F: *P. pentosaceus* S128b + *L. plantarum* S91 + *L. sakei* S15, G: *L. sakei* S15 + *S. carnosus* G109, H: *L. sakei* S15 + *L. plantarum* S91 + *S. carnosus* G109, I: *P. pentosaceus* S128b + *S. carnosus* G109 + *L. sakei* S15).

4. Conclusions

This study demonstrated that the incorporation of autochthonous starter cultures (ASCs) can play a decisive role in shaping the quality characteristics of traditionally produced sucuk under local small-scale facility conditions. The use of these strains, either as mono- or mixed-starter cultures, contributed to desirable changes in key physicochemical parameters. Moreover, the application of ASCs supported product safety by limiting the growth of undesirable microorganisms without adversely affecting lipid oxidation or water activity. In addition, groups containing *L. plantarum* were determined to be associated with a wider range of aromatic compounds, revealing their potential role in increasing the flavor complexity of sucuk. Taken together, these findings highlight the potential of ASCs as an effective tool for improving the quality and safety properties of artisanal fermented meat products, thereby providing a sustainable strategy to strengthen both product standardization and consumer appeal. However, sensory assessments are also required to confirm the impact of ASC-induced changes on consumer preference and product acceptance.

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