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Metagenomic analysis of microbial diversity in sucuk, a traditional Turkish dry-fermented sausage, and its relationship with organic acid compounds

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ORIGINAL ARTICLE

Abstract

Sucuk is a traditional Turkish fermented meat product that is widely consumed in Türkiye. The aim of this study was to determine the microbial diversity and organic acid profile and to elucidate their mutual relationship. The most abundant phylum in sucuk was Firmicutes, followed by Proteobacteria and Cyanobacteria phyla. The most abundant genera in sucuk were *Lactobacillus*, *Pediococcus*, and *Staphylococcus*. Acetic, lactic, and tartaric acids were found in all sucuk samples. Tartaric and lactic acids were positively correlated with microbial diversity parameters. Furthermore, tartaric acid was found to be an indicator of the presence of a rare genus, while lactic acid was found to be an indicator of a balanced distribution among genus and the dominance of some genus. This study for the first time showed that the microbiota of fermented Turkish sausage will be an important contribution to future studies.

Keywords: Metagenomic; Microbiota; Organic acid; Sucuk; Turkish fermented sausage

Introduction

Sucuk is a traditional dry fermented sausage that is widely consumed in Türkiye. It is typically prepared using beef/buffalo/mutton, tail fat, salt, sugar, nitrite/nitrate, as well as garlic, cumin, allspice, black pepper, and red pepper. Sucuk dough is a mixture of these ingredients and is naturally fermented by microorganisms at appropriate temperature and time (Kilic, 2009).

The fermentation process of sucuk can last up to 20 days, with initial temperatures ranging from 12 to 26°C (Kaban and Kaya, 2008). Fermentation process contributes to sucuk's flavor, aroma, and product qualities. Sucuk is characterized by its taste, which results from the complex interplay between its ingredients and microbial communities involved in fermentation (Liao *et al.*, 2022).

Lactic acid bacteria (LAB) and coagulase-negative staphylococci (CNS) are responsible for sucuk fermentation. Starter cultures are widely used in industrial production to produce the same quality product and to inhibit foodborne pathogens. Commonly used culture mixtures are anaerobic LAB, especially *Latilactobacillus*, *Lactiplantobacillus*, *Leuconostoc*, *Pediococcus*, *Lactococcus*, and *Enterococcus*, while CNS are *Staphylococcus xylosus* and *S. carnosus* (Van Reckem *et al.*, 2019).

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LAB play a crucial role in decreasing the pH of sucuk by producing lactic acid, which inhibits the growth of spoilage and pathogenic bacteria (Akköse *et al.*, 2023; Kamiloğlu *et al.*, 2019). In addition, CNS contribute to the development of flavor and the overall safety of the product by producing antimicrobial compounds (Akköse *et al.*, 2023; Kesmen *et al.*, 2012). The presence of these bacteria not only improves the microbiological safety of sucuk but also influences its organoleptic properties (Demirel and Gürler, 2018).

According to the new taxonomic classification, the lactobacilli commonly found in fermented meat products are *Companilactobacillus*, *Dellaglioa*, *Lacticaseibacillus*, *Lactiplantibacillus*, *Latilactobacillus*, and *Paucilactobacillus* (Zheng *et al.*, 2020). CNS that are frequently isolated from fermented meat products include *S. xylosus*, *S. saprophyticus*, and *S. equorum* (Kaban and Kaya, 2008). The color and flavor of sausage are affected by CNS owing to their enzymes, such as nitrate reductase, protease, and lipase. (Kaban *et al.*, 2012).

The microbiota of fermented foods can be explained by culturomics and nonculturomic approaches. Nevertheless, culturomics has its drawbacks, as it may not fully represent the entire microbial population because of organisms that are difficult to culture or cannot be cultured at all (Parmar *et al.*, 2018). In culture-based methods, microbial loss of samples occurs due to factors such as the selection of colonies with false morphological characteristics, the use of selective media, or the misinterpretation of biochemical tests.

On the other hand, a nonculturomic method such as next generation sequencing (NGS) provides an in-depth assessment of the microbial population without cultivation. Nonculturomic methods have advantages. NGS can detect microorganisms, especially those that are impossible to culture using traditional methods like researchers and different methodologies. NGS can be the vital key for optimizing fermentation processes and improving product quality (De Filippis *et al.*, 2017).

In recent studies, the microbial diversity of fermented meat products, such as Korean dry-fermented sausages (Kim *et al.*, 2022), Mediterranean spontaneously fermented sausages (Bassi *et al.*, 2022), salami-type dry-fermented sausages from Brazil (Degenhardt *et al.*, 2021), Italian sausages (Franciosa *et al.*, 2021), Fuet fermented sausages (Yang *et al.*, 2022), Felino-type sausages (Ferrocino *et al.*, 2018), artisanal fermented sausages (Barbieri *et al.*, 2021), and salami sausages (Liu *et al.*, 2023) have been identified through metagenomic analysis. However, no metagenomic study has been found on the microbiota of sucuk-dry fermented meat products.

This research aimed to unravel sucuk production processes by explaining the relationship between microbial diversity and organic acid production, ensuring food safety and increasing the final product quality. For that purpose, this study examined the diverse microbial communities found in sucuk through metagenomic analysis and also assessed the organic acid content, which plays a key role in the development of the flavor and preservation of sucuk.

Material and Methods

Samples

Ten traditionally produced and fermented Turkish sucuk (Ts) samples obtained from local markets in Isparta-Türkiye were used in this study. The sucuk produced from beef were aseptically collected in July 2023 and stored at +4°C. Sucuk samples were aseptically divided into sterile containers for analysis.

Methods

DNA extraction

DNA extraction was performed according to the method described by Liu *et al.* (2004) and modified by Ucak *et al.* (2022). In summary, 10 g of sucuk sample was homogenized in 90 mL pepton water using ultra turrax (Ika, Germany). One milliliter of homogenate was added to a centrifuge tube and centrifuged (10,000 × g for 5 min at room temperature). The pellet was treated with 0.5 mL 1× TE buffer (containing 10 µg/mL lysostaphin and 4 mg/mL lysozyme) during 18 h at 37°C. After incubation, 10% SDS and 20 mg/mL proteinaz K were added to the lysed pellets. Finally, the extracted DNA was dissolved in 70 µL sterile ultrapure water. DNA was quantified using a Qubit 4 fluorometer (Thermofisher, Finland).

Library preparation

DNA library preparation was conducted according to the library preparation procedure for 16S metagenomic sequencing (Illumina Inc.,CA,USA). The primers containing overhang adapter sequences, forward primer:5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG-3', and reverse primer:5'-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3' were used for amplicon PCR experiments. In amplicon PCR, the 16S rRNA V3-V4 regions were amplified by KAPA HiFi HS Mix (Roche, Germany). After amplicon PCR, the samples were indexed with dual indexes using the Nextera® XT index Kit v2 Set-A (Illumina). For library preparation, all amplicons and indexed samples were cleaned using AMPure XP beads

(Beckman Coulter, USA) on a magnetic rack (DynaMag™-96 Side, Invitrogen, Norway). The ratios of the samples were adjusted to equimolar amounts and subsequently diluted to a final concentration of 35 pmol DNA library. A 20 µL library containing 5% (v/v) PhiX control DNA (Illumina) was then loaded into an iSeq100 v1 cartridge (Illumina).

Metagenomic and bioinformatic analysis

Sequencing was performed using the iSeq100 system (Illumina) pair-end read type and two reads of 151 bp read length. Sequencing data were obtained using the iSeq100 system. The data were analyzed by 16S Metagenomics, V. 1.1.0 software (Illumina) following the manufacturer's instructions.

The raw sequencing data consisted of 4 million reads generated by Illumina iSeq100. Quality control (QC) was performed using RTA2, where low-quality bases (quality score < Q30) and adapter sequences were removed. After QC, reads passed the filtering criteria and were retained for downstream analysis.

For taxonomic classification, the ribosomal database project (RDP) ensured high-accuracy taxonomic assignments. Reads were aligned and classified using 16S Metagenomics, V. 1.1.0 software (Illumina) with a confidence threshold of 97% for species-level identification. The operational taxonomic units (OTUs) were clustered at 98% identity threshold using DADA2.

All steps were performed according to standard pipelines, and the workflow was validated to ensure reproducibility. Raw data were deposited in NCBI Sequence Read Archive database.

Microbial diversity parameters such as Shannon species diversity index value, Species Richness, Evenness, Chao1 Index, Berger–Parker Dominance Index, Simpson Diversity Index, and Margalef Diversity Index were determined by the Biodiversity Component (BİÇEB) Calculation Software (https://kantitatifekoloji.net/biceb).

Organic acid analysis

Organic acid extraction from the sucuk samples was performed using water. Sucuk 5 g was weighed into a tube, and 25 mL of deionized water was added. After thorough homogenization in water, the tubes were kept in a shaker at 250 rpm for 4 h. After the tubes were centrifuged at 1900 × g for 15 min, the samples were prepared for injection by passing them through a 0.45 micron filter. The organic acid composition was determined using a Shimadzu LC2040 Prominence Brand HPLC system (Tokyo, Japan) with an LC20 AT pump and DAD detector. The mobile phase was 10 mM $NH_4H_2PO_4$ (pH 2.6, H_3PO_4), the flow rate was 1 mL/min, the injection volume was 10 µL, and the column temperature was 40°C. Column oven CTO-10ASVp and InertSustain C18 5 µm 250 mm *4.6 mm column were used. The results were calculated using the LC Solution computer package program (Soyuçok, 2022).

Statistical analysis

Principal component analysis (PCA) was performed to determine the relationship between organic acids and microbial diversity in sucuk samples using the Unscrambler software (version 10.4, Oslo, Norway). For PCA, all variables were selected at an equal weight level. The full cross-validation method was used for the validation. Singular value decomposition (SVD) was selected as the model algorithm.

The distinction between bacterial diversity and organic acid compounds in the sucuk samples was visualized using a heatmapper (http://www.heatmapper.ca). The values are between –4(blue) and +4 (yellow) with zero in the middle. The rows are clustered, and the different color scale was used to clearly identify and visualize the different values on the heat map.

Results and Discussion

Microbial diversity

This study focused on species and genera that reached concentrations above 0.5% of OTUs in at least one of the samples. In total, more than 350 OTUs were identified, indicating that the sausage microbiota examined had an extremely rich biodiversity. This diversity demonstrates that microbial species vary significantly not only in numerical terms but also in their relative composition among samples.

The relative abundances of bacterial OTU belonging to the phylum, the class, the order, the family, the genus, and the species levels of sucuk samples were presented in figures. The top phylum classification results are shown in Figure 1A. The dominant phylum in the sucuk samples was Firmicutes, and it was followed by Proteobacteria, Cyanobacteria/Chloroplast, Bacteroidetes, and Actinobacteria. The top class classification results are shown in Figure 1B. In the sucuk samples, bacilli were abundant at the class level. The main order was Lactobacillales in all sucuk samples (Figure 1C). The most abundant bacterial families identified were Lactobacillaceae, Staphylococcaceae, and Leuconostocaceae in sucuk samples (Figure 1D).

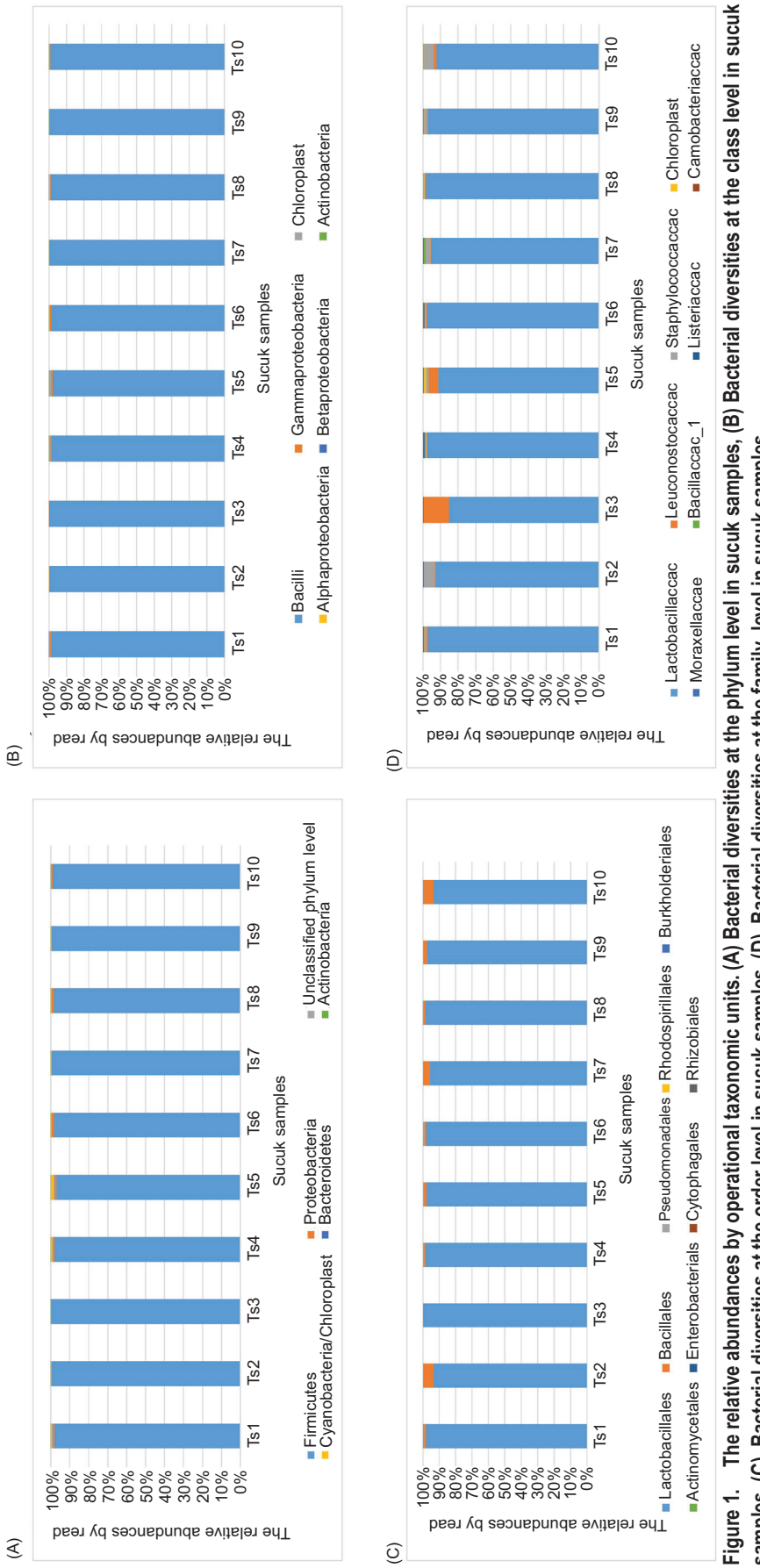


Figure 1. The relative abundances by operational taxonomic units. (A) Bacterial diversities at the phylum level in sucuk samples, (B) Bacterial diversities at the class level in sucuk samples, (C) Bacterial diversities at the order level in sucuk samples, (D) Bacterial diversities at the family level in sucuk samples.

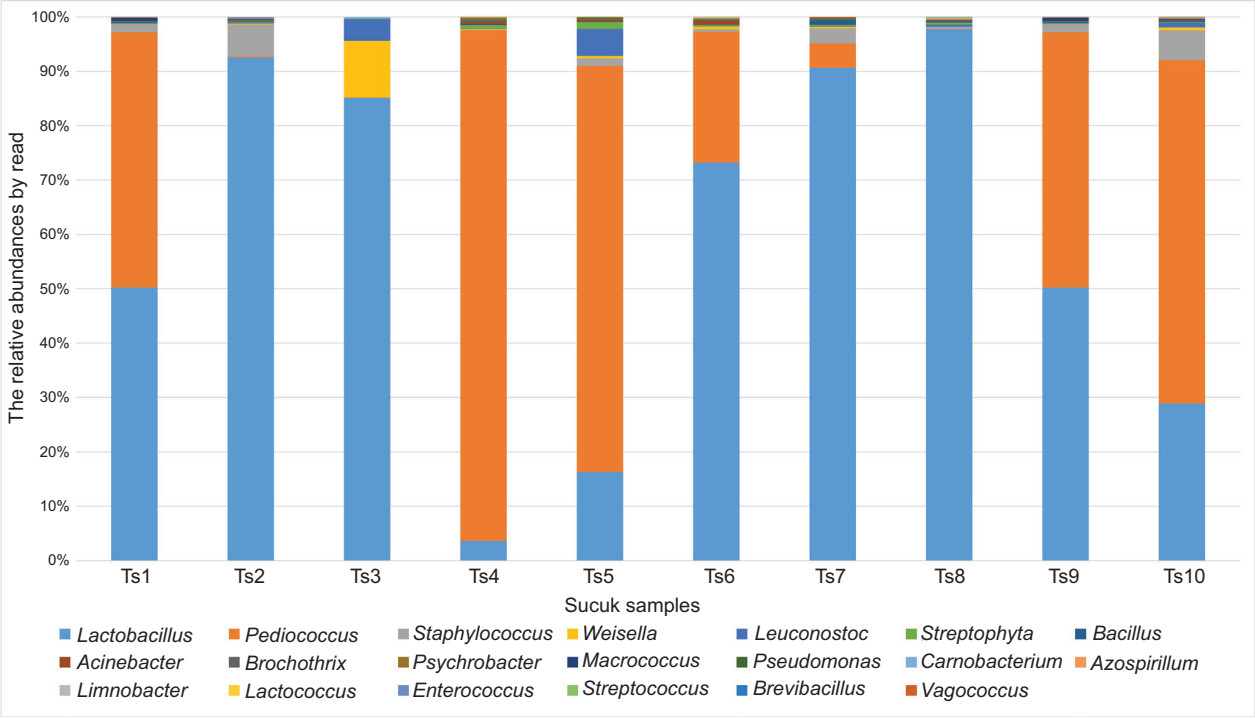


Figure 2. Bacterial diversities at the genus level in sucuk samples.

At the genus level, *Lactobacillus* genus was dominant in Ts2, Ts3, Ts6, Ts7, and Ts8 samples (Figure 2). *Pediococcus* was also the dominant genus in Ts1, Ts4, Ts5, and Ts10 samples. Ts9 was the only sucuk sample in which *Lactobacillus* and *Pediococcus* densities were balanced (Figure 2).

Analysis of bacterial diversity at the genus level in sucuk samples revealed significant variations (Table 1). The Shannon species diversity index fluctuated between 0.186 (Ts8) and 0.978 (Ts10). Richness values showed considerable differences, with Ts3 exhibiting the lowest at 38 and Ts10 the highest at 102. Evenness, which quantifies species distribution uniformity, ranged from 0.045 (Ts8) to 0.217 (Ts5). The Chao1 index, estimating species richness, spanned from 47.43 (Ts3) to 210.10 (Ts10).

The Berger–Parker dominance index demonstrated the lowest dominance in Ts8 (1.026) and the highest in Ts9 (1.995). The Simpson diversity index, measuring the likelihood of two individuals belonging to identical species, varied from 0.050 (Ts8) to 0.529 (Ts9). The Margalef diversity index, which reflects species richness in relation to sample size, ranged between 3.244 (Ts3) and 8.807 (Ts10). In addition, the Menhinick diversity index, another measure of species richness, spanned from 0.127 (Ts3) to 0.368 (Ts4). These results underscore the variability in bacterial composition and richness among the analyzed sucuk samples.

According to diversity parameters, such as Shannon species diversity index, Evenness, Berger–Parker Dominance Index, Simpson Diversity Index, Margalef Diversity Index, and Menhinick Diversity Index, the balanced and highest microbial diversity at the genus level was found in Ts5, Ts6, Ts9, and Ts10 (Table 1). It is seen in Figure 2 that no dominant species was found in the Ts9 sample, and this is supported by Table 1. The Berger–Parker Dominance Index and Simpson Diversity Index and Evenness values of Ts2, Ts4, and Ts8 samples were found to be very weak in terms of microbial diversity, as they had low values (Table 1, Figure 2). The microbial diversity parameter values of Ts1, Ts3, and Ts7 samples were very poor in terms of microbial diversity, as they had moderated values (Table 1).

The dominant bacterial species was *P. pentosaceus* in Ts1, Ts5, and Ts6 samples (Figure 3). For Ts4, Ts7, Ts9, and Ts10 samples, *P. lolii* was found to be the major species. *Llb. graminis* was found to be the dominant species in Ts3 and Ts 8 samples. *S. xylosus* was only found as a major bacterial species in Ts2 sample (Figure 3).

According to Evenness and Berger–Parker Dominance Index, one species was found to be dominant in Ts1, Ts3, Ts4, and Ts9 sucuk samples, and the species found were *P. pentosaceus*, *Llb. graminis*, *P. Lolii,* and *P. pentosaceus*, respectively (Figure 3). The balanced and highest microbial diversity at the species level was observed in Ts2, Ts7, and Ts8 samples (Table 2). The microbial diversity

Table 1. The bacterial diversity parameters at the genus level in sucuk samples.

Sample Name	Shannon species diversity index[a]	Richness[b]	Evenness*	Chao1 Index	Berger–Parker Dominance Index	Simpson Diversity Index	Margalef Diversity Index	Menhinick Diversity Index
Ts1	0.448	63	0.108	79.50	1.104	0.176	5.580	0.244
Ts2	0.355	60	0.087	110.75	1.082	0.143	5.387	0.251
Ts3	0.543	38	0.149	47.43	1.176	0.264	3.244	0.127
Ts4	0.360	94	0.079	160.11	1.069	0.123	8.391	0.368
Ts5	0.891	61	0.217	76.55	1.342	0.416	5.520	0.266
Ts6	0.760	70	0.179	132.00	1.371	0.410	6.328	0.300
Ts7	0.461	67	0.110	139.50	1.104	0.177	5.827	0.233
Ts8	0.186	62	0.045	108.20	1.026	0.050	5.431	0.226
Ts9	0.868	61	0.211	91.60	1.995	0.529	5.492	0.259
Ts10	0.978	102	0.211	210.10	1.588	0.518	8.807	0.330

*: Evenness= a/ln(b).

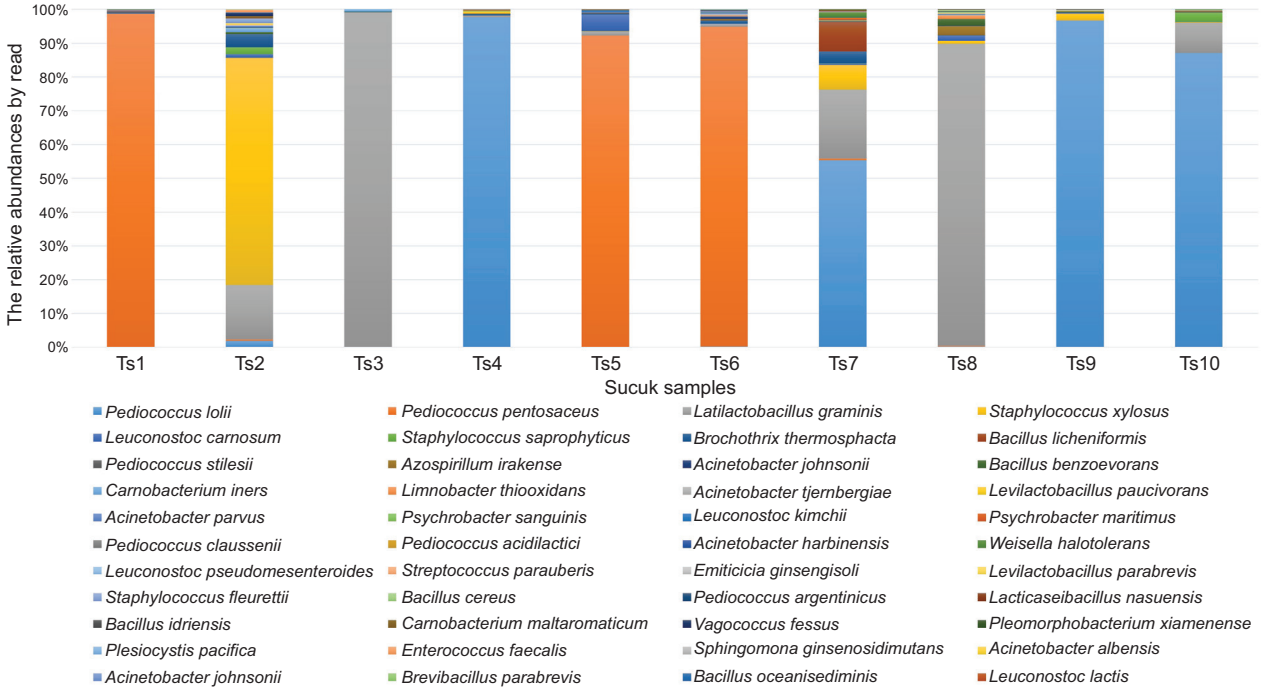


Figure 3. Bacterial diversities at the species level in sucuk samples.

parameter values of Ts5, Ts6, and Ts10 samples were poor in terms of microbial diversity, as they were average values (Table 2).

Traditional sucuk microflora include a variety of microorganisms, especially LAB, molds, and yeasts. These organisms play key roles in sucuk fermentation and ripening. Species such as *Lactobacillus*, *Staphylococcus*, and *Micrococcus* have a significant effect on this process. LAB reduce the pH of sucuk by producing lactic acid and bacteriocins and inhibiting the growth of pathogenic organisms. Simultaneously, it contributes to the emergence of various sensory properties by modifying the raw material (Nazlı *et al.*, 2017). Typically, the pH level in dry fermented sausages ranges from 4.5 to 5.5 (De Mey *et al.*, 2017). Sucuk also falls within this range, with a pH value of 5.4 or lower (Anonymous, 2019). As stated by Özdal (2020), these positive effects of LAB improve the overall quality of sucuks and contribute to improving their taste, color, and texture.

In traditionally fermented meat products, fermentation occurs spontaneously, or the natural microbial flora in

Table 2. The bacterial diversity parameters at the species level in sucuk samples.

Sample Name	Shannon species diversity index value[a]	Richness[b]	Evenness*	Chao1 Index	Berger–Parker Dominance Index	Simpson Diversity Index	Margalef Diversity Index	Menhinick Diversity Index
Ts1	0.15	97	0.033	145.23	1.019	0.038	8.717	0.394
Ts2	1.53	83	0.346	143	1.553	0.558	9.955	1.350
Ts3	0.098	66	0.023	102.25	1.013	0.025	6.110	0.323
Ts4	0.22	142	0.044	227.45	1.029	0.056	12.788	0.573
Ts5	0.49	98	0.107	167.46	1.105	0.178	9.103	0.476
Ts6	0.47	109	0.100	208	1.073	0.131	11.338	0.931
Ts7	1.65	118	0.346	194	1.873	0.662	13.275	1.439
Ts8	0.806	90	0.179	168.3	1.160	0.255	9.861	0.987
Ts9	0.273	111	0.058	205	1.042	0.078	10.798	0.681
Ts10	0.56	149	0.112	282	1.152	0.239	13.303	0.572

*: Evenness= a/ln(b).

the environment is influenced by various parameters, such as raw material, spices, fermentation temperature, and duration (Stavropoulou *et al.*, 2018a). Cinar *et al.* (2018) reported that *Lpb. plantarum* was dominant in fermented sausages. In a similar study, *Lpb. plantarum* strains found in sucuk were reported to have antagonistic activity against *S. aureus*, *Listeria monocytogenes*, and *Bacillus cereus* (Kamiloğlu *et al.*, 2020). It has been suggested that the bacteriocin produced by *Llb. curvatus* isolated from fermented meat products inhibits *L. monocytogenes* and is therefore used in the production of fermented meat products (Casaburi *et al.*, 2016). The use of *Llb. curvatus*, *Llb. Sakei*, and *Lpb. plantarum* in fermented meat products reduces the amount of biogenic amines in sucuk (Doğan *et al.*, 2020).

The use of high amounts of salt increases the growth of CNS, but differences in salt levels (2–4%) do not affect the biodiversity of CNS (Charmpi *et al.*, 2020; Van Reckem *et al.*, 2019). The low temperature fermentation process with natural microflora increases the growth of *S. equorum*, *S. Saprophyticus*, and *S. xylosus*, while the high temperature enhances the growth of *S. lugdunensis* and *S. aureus* (Charmpi *et al.*, 2020; Stavropoulou *et al.*, 2018b).

In Ts1, Ts3, Ts4, Ts6, Ts8, and Ts9, most of the ASVs were attributed to a single species, and the other samples were characterized by higher biodiversity, with an important diversification in the composition of microbiota. Among LAB, *Llb. graminis* was the major species (>80% of ASVs in Ts3 and Ts8). The *Llb. sakei* group divides into four species, renamed after the new taxonomy. These are *L. sakei*, *L. graminis*, *L. curvatus*, and *L. fuchuensis* (could not be discriminated using 16S rRNA sequence) (Zheng *et al.*, 2020). These two types of microorganisms were expressed as *L. sakei/L. graminis* together in a study in which microbial diversity in the pastırma was determined using the fingerprint method (Metin and Toy, 2023). Therefore, the results of this study were compared with those of *L. sakei* reported in the literature. Although LAB species diversity in fermented meat products was limited, *L. sakei* was predominant during the ripening process, due to the species' excellent adaptation, competitiveness, and assertiveness in the meat medium (Aquilanti *et al.*, 2016; Janßen *et al.*, 2018; Stavropoulou *et al.*, 2018b). This superiority over other LAB can be attributed to its salt-tolerant and psychrotrophic nature and the use of the arginine deiminase pathway and nucleosides in the meat environment (Fontana *et al.*, 2016).

Organic acid components of sucuk samples

The organic acid compositions are listed in Table 3. Tartaric acid, lactic acid, and acetic acid were found in all sausage samples (Table 3). The lowest level of tartaric acid was found in the Ts1 sample at 3.22 mg/g and the highest level was found in the Ts9 sample at 6.00 mg/g. Lactic acid was lowest in the Ts2 sample at 1.28 mg/g and highest in the Ts9 sample at 3.59 mg/g. The lowest level of acetic acid was found in the Ts1 sample at 0.72 mg/g; the highest level was found in the Ts10 sample at 3.89 mg/g. Oxalic acid was detected in Ts4, Ts5, Ts6, Ts7 and Ts9 at 0.23, 0.17, 1.62, 0.15, and 0.22 mg/g, respectively. Malonic acid was found only in the Ts1 (2.01 mg/g) and Ts2 (2.78 mg/g) samples. Citric acid was highest in Ts1 (1.91 mg/g), followed by Ts5 (0.93 mg/g), Ts2 (0.71 mg/g), Ts3 (0.45 mg/g), and Ts7 (0.41 mg/g). Succinic acid was found in all samples, except Ts1 and Ts2. Propionic acid was found at 27.09, 19.81, 35.50, 39.52, and 35.03 mg/g in Ts1, Ts2, Ts3, Ts4, and Ts5 samples, respectively.

Table 3. Organic acid content of sucuk samples.

Sample	Organic acid (mg/g)							
	Oxalic acid	Tartaric acid	Malonic acid	Lactic acid	Acetic acid	Citric acid	Succinic acid	Propionic acid
Ts1	n.d	3.22	2.01	1.35	0.72	1.91	n.d	27.09
Ts2	n.d	4.01	2.78	1.28	1.03	0.71	n.d	19.81
Ts3	n.d	5.64	n.d	2.02	2.18	0.45	7.63	35.50
Ts4	0.23	4.18	n.d	2.30	2.65	n.d	8.55	39.52
Ts5	0.17	4.34	n.d	3.06	2.65	0.93	2.47	35.03
Ts6	1.62	4.32	n.d	2.43	1.26	n.d	6.07	n.d
Ts7	0.15	3.72	n.d	1.84	3.08	0.41	6.51	n.d
Ts8	n.d	3.68	n.d	2.36	1.60	n.d	6.88	n.d
Ts9	0.22	6.00	n.d	3.59	2.99	n.d	9.27	n.d
Ts10	n.d	5.80	n.d	2.80	3.89	n.d	7.38	n.d

n.d: not detected.

Carbohydrate fermentation in fermented meat products produces organic acids that affect the texture and sensory properties of the final product by lowering its pH. Acid formation depends on the type and concentration of sugars present, sheath diameter, and other technological factors, particularly the type of bacteria (Bangar *et al.*, 2022). During the fermentation and drying/maturation stages, LAB utilize the sugars added to fermented meat products, resulting in the formation of lactic acid, which is the primary organic acid responsible for the decrease in pH of fermented meat products. The use of sugar in these products inhibits pathogenic and spoilage bacteria because of the desired decrease in pH and contributes to the typical organoleptic character of the product (Hwang *et al.*, 2023).

The production of organic acids affects the sensory properties, such as the taste and aroma of sucuk (Laranjo *et al.*, 2019). Fermentation parameters such as temperature, ingredients, and fermentation conditions significantly change microbial diversity, leading to variations in organic acids and thus the overall flavor and preservation characteristics of sucuk (Baka *et al.*, 2011). The presence of specific LAB strains can enhance desirable organic acid production, further contributing to the flavor and safety of the product by inhibiting undesirable microbial growth (Laranjo *et al.*, 2019). Besides lactic acid, other organic acids, including acetic and propionic acids, may also be present in sucuk. These acids can be produced as secondary metabolites during fermentation and contribute to the overall flavor profile of sausages (Nediani *et al.*, 2017).

Organic acids in fermented meat products are typically classified into two groups, with lactic acid, succinic acid, and acetic acid being the desired acids, and citric, malonic, pyruvic, formic, butyric, and propionic acids being the undesirable acids that should not exceed certain levels (Erginkaya, 1993). This classification is based on the fermentation method used, with the desired acids being formed homofermentatively and the undesired acids being formed heterofermentatively (Erginkaya, 1993). Organic acids are formed by the fermentation of sugars, acetic acid is also formed through fatty acid oxidation and alanine catabolism, and propionic and butyric acid are formed through the oxidation of aldehydes (Ravyts *et al.*, 2012). Production temperature, starter cultures, and sugar content are the main factors that affect acid formation during the fermentation of meat products (Halagarda and Wójciak, 2022). In production, it is important to determine the ideal temperature and acidity level by considering product-specific acidity levels and microbial flora.

Relationship between microbial communities and organic acids

Eighty percent of the total variance is explained by PC1 and PC2 (Figure 4A). The percentage of variance explained by PC1 and PC2 was 52% and 28%, respectively. Only oxalic acid is located on the positive PC1 line. All the other loadings were located on the negative PC1 line (Figure 4B). All bacterial genera, such as *Psychrobacter*, *Lactobacillus*, *Acinetobacter*, *Streptophyto*, *Streptococcus*, and *Bacillus*, and some organic acids (lactic acid, acetic acid, and succinic acid) were the variables with the highest contribution to the PC (Figure 4B). A 50% variance was explained by genera, such as *Carnobacterium*, *Leuconostoc*, *Weisella*, and oxalic acid. Thus, these loadings did not contain enough structured variants to be discriminated against in sucuk samples. The loadings in the outer ellipse explained 100% of the variance (Figure 4C).

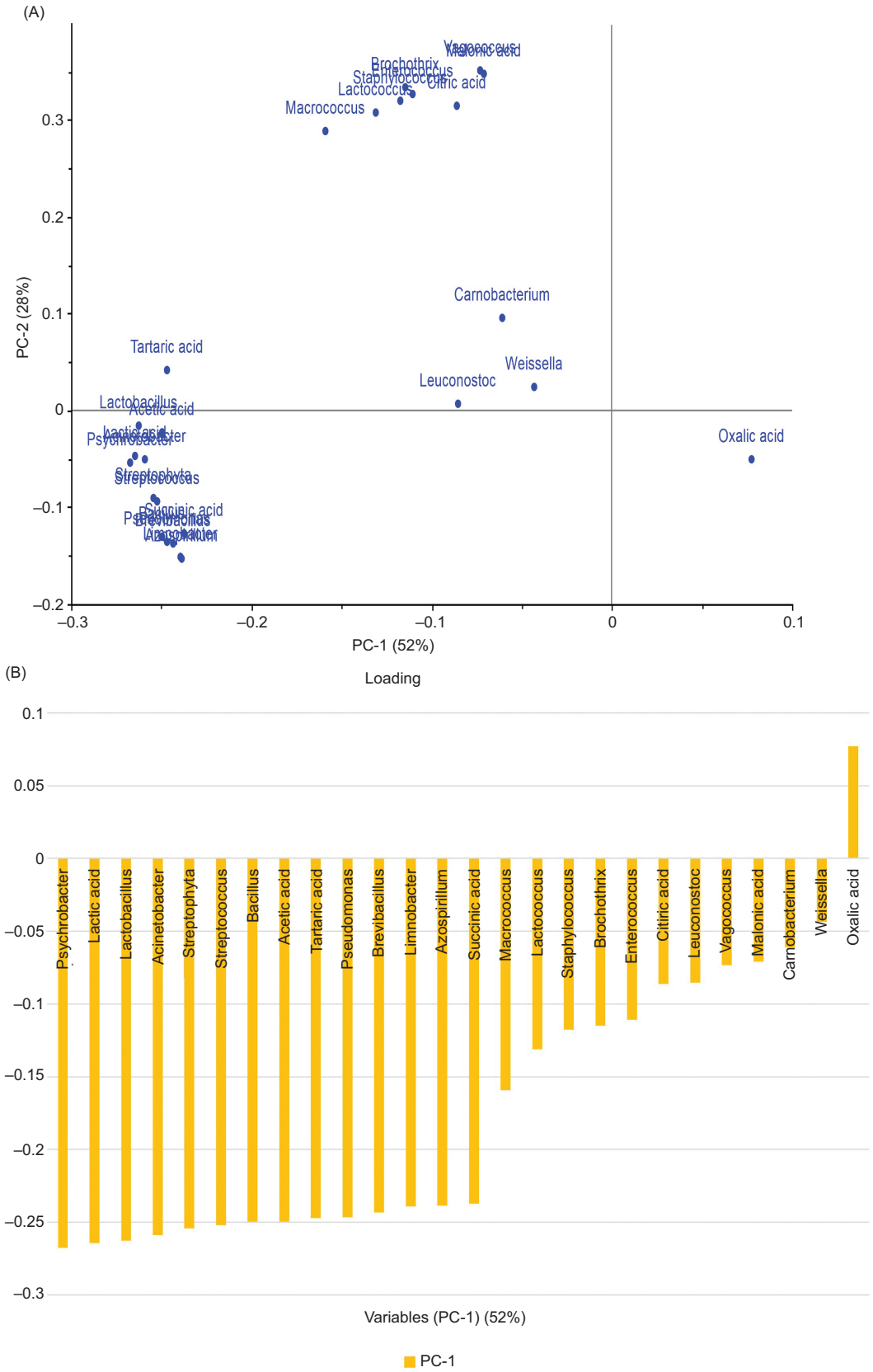


Figure 4. (A) The effects of PC on total variance. (B) The contribution of loadings to PC 1.

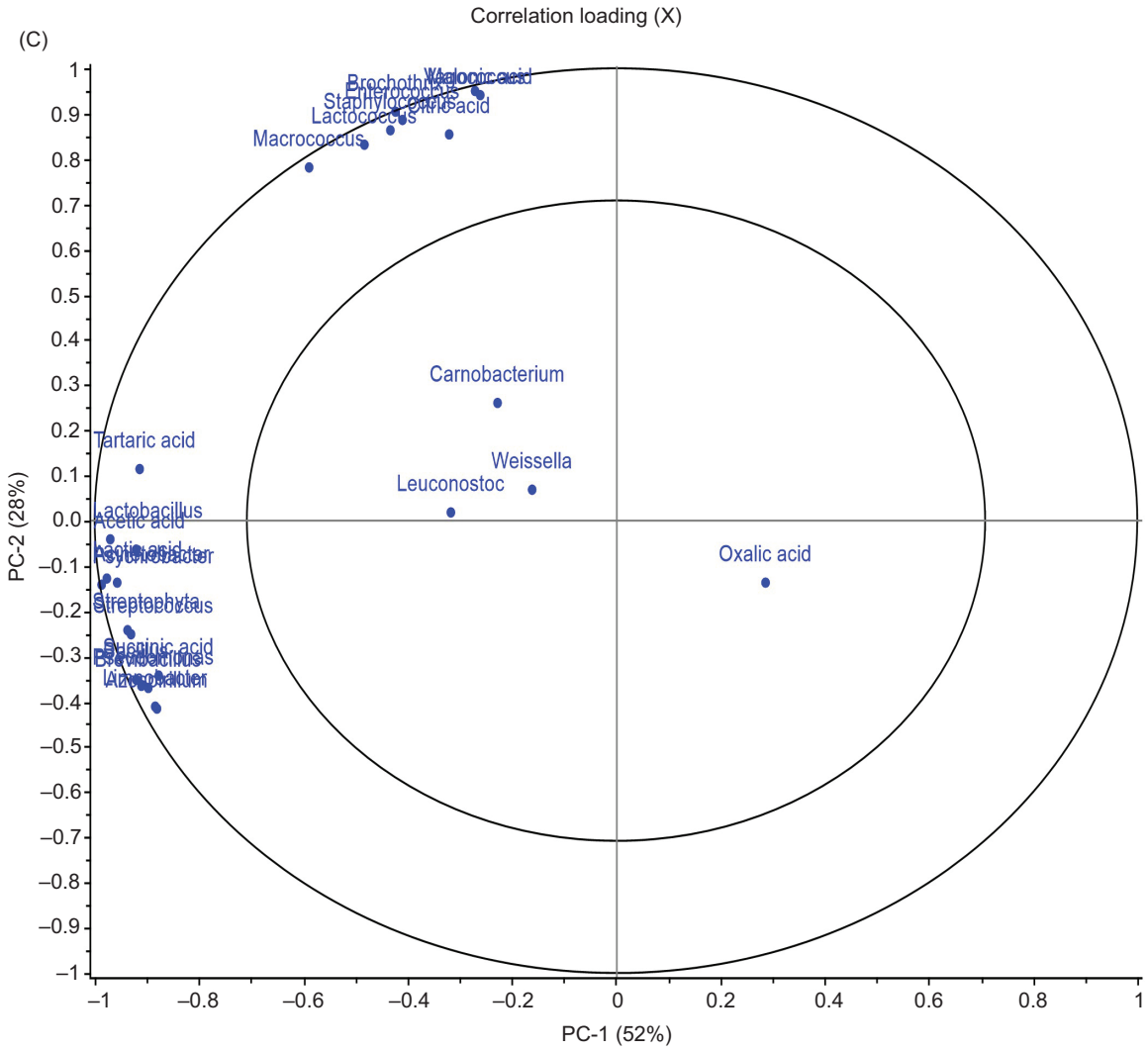


Figure 4. (Continued) (C) Correlation loadings of relationships between organic acid and microbial diversity at the genus level.

Ts2, Ts3, and Ts8 were separated from the other sucuk samples in terms of microbial diversity and organic acid compounds by PC1 (Figure 5). The heatmap also supports these results (Figure 6). The heat map shows two major clusters (Figure 6). The first subunit of the first cluster contained only Ts9. The second subunit of the first cluster consisted of Ts6, a subbranch of Ts2-Ts3, and a subbranch of Ts7-Ts8. The first subunits of the second cluster were the Ts5 and Ts4-Ts1 branches. The second subunit of the second cluster consisted of only Ts10. Characteristic differences in the sucuk samples were revealed using both PCA and heat map.

The correlation results between the organic acid and microbial diversity parameters at the species level are given in Table 3. According to the results, the correlation between tartaric acid and richness and Chao1 index was significant at 0.05 level (r=0.646 and 0.691, respectively). The highest correlation between lactic acid and microbial diversity parameters was found in Berger–Parker Dominance Index (r=0.801) at 0.01 level. In addition, lactic acid and evennes (r=0.686), Shannon species diversity index (r=0.693), and Simpson Diversity Index (r=0.735) values were correlated at 0.05 level. NGS was used to identify the microbial structures of sucuks to overcome the limitations in identifying bacterial populations. This study is unique in that it is the first study on sucuk. The presence of abundant bacteria at the phylum, the genus, and the species levels was identified. The abundant phylum was Firmicutes. The most species were *Lactobacillus*, *Pediococcus*, and *Staphylococcus*. *P. loli*, *P. Pentosaceus*, and *Llb. graminis* were the dominant species. Lactic acid, tartaric acid, and acetic acid were commonly found in sucuks. When the relationship between organic acids and microbial diversity parameters was analyzed, it was found that only tartaric acid and lactic acid were positively

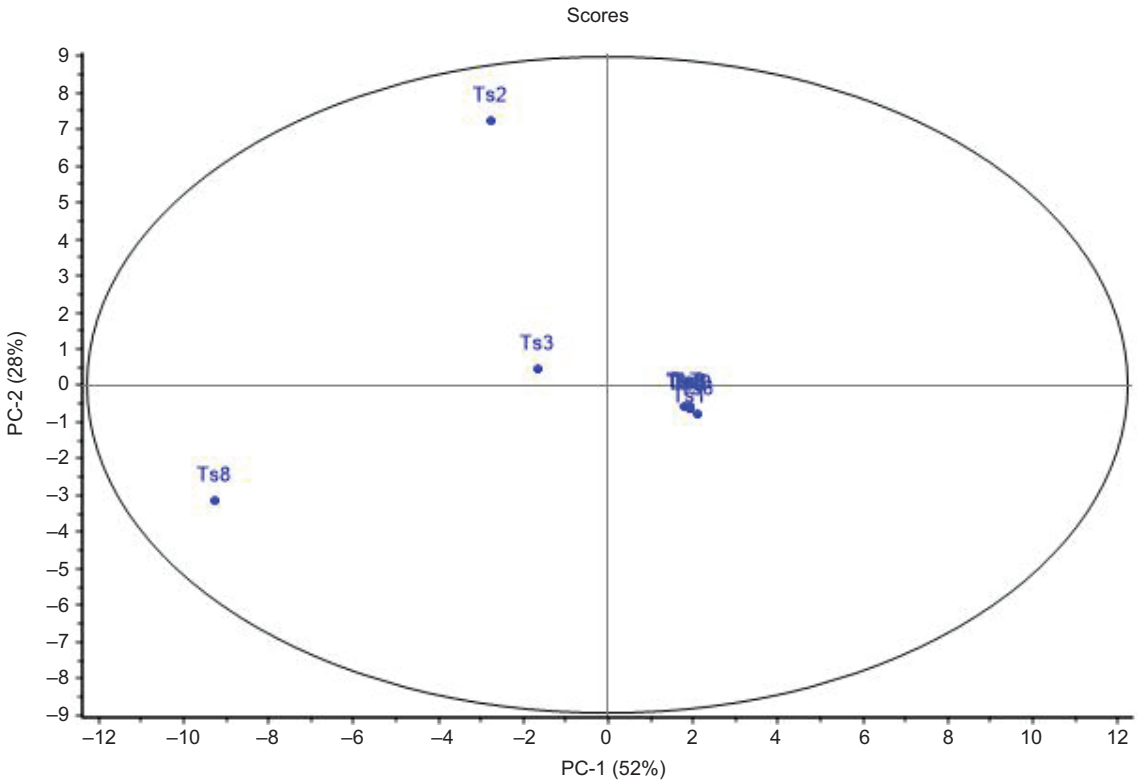
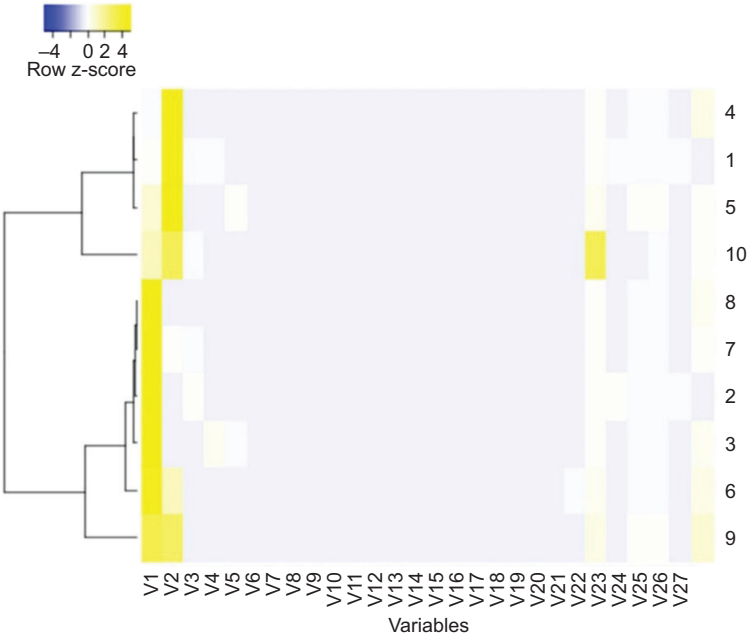


Figure 5. The results of PC1 versus PC2 of sucuk samples.



V1: *Lactobacillus*, V2: *Pediococcus*, V3: *Staphylococcus*, V4: *Weissella*, V5: *Leuconostoc*, V6: *Streptophyta*, V7: *Bacillus*, V8: *Acinetobacter*. V9: *Brochothrix*, V10: *Psychrobacter*, V11: *Macrococcus*, V12: *Pseudomonas*, V13: *Carnobacterium*, V14: *Azospirillum*, V15: *Limnobacter*, V16: *Lactococcus*, V17: *Enterococcus*, V18: *Streptococcus*, V19: *Brevibacillus*, V20: *Vagococcus*, V21: *Oxalic acid*, V22: *Tartaric acid*, V23: *Malonic acid*, V24: *Lactic acid*, V25: *Acetic acid*, V26: *Citric acid* and V27: *Succinic acid*

Figure 6. Cluster analysis of a heat map showing the relationship between organic acid and microbial diversity at the genus level.

correlated with some parameters. Tartaric acid was generally correlated with parameters indicating the presence of rare species. Lactic acid, on the other hand, was found to be correlated with the parameters that dominate ordam and indicate a balanced distribution of microbial diversity. It may also suggest that lactic acid increases the imbalance between species in the ecosystem and causes a certain species to dominate over others. Particularly high concentrations of lactic acid may have negative effects on species richness and balance in the ecosystem, as a dominant species may suppress other species or cause them to compete. The comprehensive metagenomic analysis conducted with sucuk can enlighten the microbial structures of the products in different regions of the world and strengthen their applications in the food industry.

Data Availability Statement

Raw sequence data obtained in this study using next generation sequencing were submitted to the NCBI Sequence Read Archive database with BioProject accession number PRJNA1195933.

Authors Contribution

Ali Soyuçok: Conceptualization, data curation, formal analysis, investigation, methodology, software, validation, visualization, and writing original draft. The author read and approved the final manuscript.

Conflicts of Interest

The author declare no conflict of interest.

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