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Effect of Ultrasonication on Texture Profile Analysis, Microbiome Phylogeny, and Prediction of Fatty Acid Metabolic Pathways in Siahmazgi Cheese

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Abstract

Siahmazgi cheese ripens through the metabolic activities of the indigenous microbiota present in unpasteurized milk. To ensure its safety, non-thermal processing methods such as ultrasound are required. This study aimed to determine the textural changes and the phylogeny relationships of the microbiome in Siahmazgi cheese affected by ultrasonication, followed by a metabolomics analysis of fatty acids (FAs). Microscopic changes in cheese texture affected by ultrasonication (0, 5, 10 min) were examined through processing of SEM images by ImageJ. Texture profile analysis was also performed using a texture analyzer. Phylogenetic and metabolomics analyses of the microbiome were carried out using Geneious Prime. Correlations between microbes, metabolites, and metabolic pathways using Cytoscape. Phylogenetic analysis showed that the microbiome of cheeses treated with the same sonication exhibited similar genetic heatmaps until the third month, while the non-sonicated sample at the sixth month of ripening displayed the highest biodiversity. *Staphylococcus equorum*, *Acinetobacter johnsonii*, *Lactobacillus zeae*, *Macrococcus caseolyticus*, *Leuconostoc mesenteroides*, and *Lactiplantibacillus plantarum* were identified as key contributors to FAs metabolism. Hexadecanoic acid, octadecanoic acid, (9Z,12Z,15Z)-octadecatrienoic acid, and (9Z)-hexadecenoic acid were identified as the main FAs from lipid metabolism, and the biosynthesis of unsaturated FAs. Texture of samples prepared from 5 min-sonication showed the highest pore number (2345) and porosity (0.046352). Hardness, adhesiveness, cohesiveness, gumminess, and chewiness were the lowest in 5-min sonicated samples and the highest in 10-min sonicated samples during ripening. If a porous soft texture in the Siahmazgi cheese is desired, sonication for 5 min is recommended. Additionally, the results of this study provide a comprehensive understanding of the effect of sonication on texture, microbiome, and the prediction of metabolic pathways of FAs in Siahmazgi cheese, which supportthe development of non-thermal technologies for traditional cheeses.

Keywords: Metabolomics, Siahmazgi cheese, Texture profile analyzer, Ultrasonication

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Introduction

Siahmazgi cheese, a traditional dairy product from northern Iran, is distinguished by its rich microbial biodiversity, unique texture, characteristic flavor, and high nutritional value. Its natural fermentation, driven by milk indigenous microorganisms, leads in production specific biochemical compounds that set it apart from industrially manufactured cheeses (Fox, Guinee, Cogan, & McSweeney, 2017; Montel *et al*., 2014). Siyahmazgi cheese is a semi-hard, high-fat cheese traditionally prepared in the village of Siyahmazgi in Gilan province. It was originally made from sheep's and goat's milk, but cow's milk is now also used. This cheese undergoes prolong maturation period for six months in a sheepskin, which gives it unique physicochemical and textural properties. However, as Siahmazgi cheese is traditionally made from raw milk without pasteurization, it is susceptible to contamination by pathogenic and spore-forming bacteria, including Clostridium species, which are commonly found in artisanal cheeses (Brändle, Domig, & Kneifel, 2016; Lopez- Brea, Gómez- Torres, & Arribas, 2017). Thermal treatments, while remain the most effective means of reducing microbial risk, can adversely affect the native microbiota responsible for fermentation, potentially altering the cheese's texture, flavor, and aroma (Beresford & Williams, 2004; Settanni & Moschetti, 2010). Non-thermal technologies, particularly ultrasonication, have emerged as promising approaches to enhance the safety and quality of dairy products without compromising their sensory or functional properties. Ultrasonication, through acoustic cavitation, can modify protein networks, enzyme activity, microbial composition, and metabolic pathways in cheese production (Feng, Barbosa-Cánovas, & Weiss, 2011). Changes in microbial composition can affect fatty acid (FA) metabolism, including synthesis and degradation, which in turn influence sensory and functional characteristics. Integrating phylogenetic analysis with metabolomics enables detailed assessment of microbial relationships, identification of FA-producing bacteria, and prediction of associated metabolic pathways.

Previous studies have investigated the effects of ultrasound at frequencies of 20, 40 and 60 kHz for 20 minutes on ultrafiltered and pasteurized Iranian white cheese (Jalilzadeh, Hesari, Peighambardoust, & Javidipour, 2018). In addition, the effect of using *Streptococcus thermophilus*, whose cell walls were lysed by ultrasonication, in the production of Cremoso cheese was also investigated (Peralta, Bergamini, & Hynes, 2019). Another example involves conducting ultrasound pretreatment of milk containing probiotic *Lactobacillus plantarum*. Results of this study indicated that, ultrasound treatment improved the growth and metabolic activity of the probiotic bacteria, leading to enhanced antioxidant activity in the final product. The ultrasound treatment increased cell membrane permeability and significantly improved the fermentation process, resulting in higher quality fermented milk (Gholamhosseinpour & Hashemi, 2019). High-intensity ultrasound leads to enhance texture and stability of products like yogurt and cheese (Guimarães *et al.*, 2018). The ultrasonication times of 5 and 10 minutes were selected based on preliminary trials and previous reports indicating that short‑ to moderate‑duration ultrasound is sufficient to induce structural modifications in dairy matrices without causing excessive protein denaturation or fat coalescence (Carrillo-Lopez *et al.*, 2020). Very short treatments (<3 minutes) do not typically generate measurable microstructural changes, while prolonged exposure (>12–15 minutes) may lead to overheating, loss of moisture, or undesirable textural defects (Jalilzadeh *et al*., 2018). Therefore, 5 and 10 minutes were chosen as representative moderate intensities capable of altering the cheese microstructure while maintaining product integrity and technological feasibility.

This study builds on prior research by evaluating the effects of ultrasonication on the texture,

microbiome phylogeny, and FA metabolism of Siahmazgi cheese. The findings provide insights into non-thermal processing for improving product quality, supporting standardization, and guiding industrial-scale production while preserving the traditional characteristics of this unique cheese.

Materials and Methods

Cheese Preparation

Siahmazgi cheese was produced using the traditional method commonly practiced in Siahmazgi village, Gilan Province. Sheep's milk collected by local nomads in Asadabad (Hamadan, Iran) was filtered and, for treated samples, sonicated (VCX 750, 22.5 kHz, 750 W, titanium probe) for 10 min before enzyme addition. The milk was heated to 35 °C, rennet (0.004%) was added, and then it was heated to 55–60 °C for about 15 min until curd formation. The curd was kneaded to remove whey, drained at room temperature for 24 h, then placed in container and covered with pasteurized whey containing 10% salt. The cheese was finally ripened at 10 °C and 40% humidity for 6 months (Jalilzadeh *et al*., 2018). The different cheese treatments, affected by ultrasonication and ripening time, included: A (0 min ultrasound, 0 months ripening), AB (5 min, 0 months), B (10 min, 0 months), C (0 min, 90 days), CD (5 min, 90 days), D (10 min, 90 days), E (0 min, 180 days), EF (5 min, 180 days), and F (10 min, 180 days) were prepared. The microbiome analysis was performed on the samples treated for 0, and 10 minutes of ultrasonication, while the other analyses were carried out on the samples treated for 0, 5 and 10 minutes of ultrasonication.

Phylogeny Analysis of Microbiomes and Metabolomics Analysis of FAs

16S rRNA metagenomic analysis was performed by extracting total DNA from cheese samples, followed by amplification of the V3–V5 region. To examine the effect of ultrasonication treatment on microbiome-mediated FA metabolism, general associations between dominant bacterial taxa and FAs were first determined. Metabolomic enrichment analyses were subsequently performed to evaluate how bacterial communities at different ripening stages and ultrasonic treatment influenced lipid-related metabolic pathways. Microbial functional prediction was conducted using PICRUSt via the Microbiome Analyst platform (Chong, Liu, Zhou, & Xia, 2020; Dhariwal *et al*., 2017; Lu *et al*., 2023). Microbiome data were filtered based on variance and prevalence, normalized using total sum scaling (TSS), and left unrarefied. Metabolomics data were processed without filtering or normalization due to a limited number of features, and auto-scaling was applied before integration. Integrated analyses included correlation heatmaps, Procrustes analysis, and metabolic network construction. Associations between microbial features and experimental metadata were assessed using general linear models, with MaAsLin2 applied to microbiome data and Limma for metabolomics data (Chong *et al*., 2020; Lu *et al*., 2023). Microbiome and metabolomics datasets were mapped onto the KEGG metabolic network to identify enriched lipid-related pathways. Microbe–metabolite correlations were visualized using interactive correlation heatmaps based on distance correlation, with defined thresholds for correlation strength and significance (Prediction potential score = 0.05; differential significance = 0.05; AGORA database). Fatty acid–microbe–pathway networks were constructed and visualized using Cytoscape v3.9.1 to identify highly connected taxa and metabolic pathways. Procrustes analysis was conducted by ordinating microbiome and metabolomics datasets into a shared multivariate space and optimally rotating and scaling configurations to assess structural alignment between microbial community composition and FA metabolic profiles. Fat was extracted from cheese samples using a 2-propanol-based method and separated by rotary evaporation. The extracted fat was transesterified with hexane and methanolic potassium to produce fatty acid methyl esters

(FAMEs). FAMEs were analyzed by gas chromatography (GC-FID), identified using retention times of standards, and reported as percentages of total fatty acids (Gholamhosseinpour & Hashemi, 2019).

Microscopic Changes in Cheese Texture

The microstructure of cheese samples at the end of the ripening period was examined using a scanning electron microscope (SEM) (FEI Quanta 450 FEG, FEI Company, Hillsboro, OR, USA). Cheese cubes were taken from the central part of the blocks, fixed and dehydrated according to standard protocols, and then mounted and gold-coated prior to imaging. The SEM images were analyzed using ImageJ software 1.40g (Java 1.6.0-05).

Texture Profile Analysis (TPA)

TPA was performed using an STM-series texture analyzer (Santam Engineering Design Co. Ltd., Tehran, Iran). Cheese samples were cut into rectangular segments (approximately 2 × 2 × 2 cm) and equilibrated to room temperature (25 ± 2 °C) before testing. A cylindrical probe (50 mm diameter) was used in step test mode. Test conditions included sample type (rectangular prism), sample thickness (2 cm), sample weight (approximately 6 g), initial distance (150 mm), test speed (60 mm/min), number of steps (4), and activation of the "Link steps" option. Compression steps were set to 6 mm, 0 mm, 6 mm, and 0 mm, respectively.

Statistical Analysis

All TPA data and porosity (image-derived parameters) were performed in triplicate for each treatment and ripening time. Data are expressed as mean ± standard deviation (n = 3). All TPA and porosity data were analyzed using two-way analysis of variance (ANOVA), considering ultrasonic treatment time and ripening time as fixed factors, followed by Tukey's post hoc test to compare means. Statistical analyses were carried out using SPSS software (IBM SPSS Statistics, version 22, IBM Corp., Armonk, NY, USA). Differences were considered statistically significant at $p < 0.05$.

Results and Discussion

Phylogenetic Analysis of Microbiomes in Siahmazgi Cheese

The genetic heat maps in Fig. 1 (a1, b1, c1, d1, e1, f1) display the alignment of 16S rRNA gene sequences among the OTUs identified in the microbiomes of various Siahmazgi cheese samples. Structural similarity within the microbiome refers to the presence of identical or closely related species, as well as similar microbial diversity in terms of the type and number of microorganisms. During the first three months of ripening, the observed microbiome similarity among samples made from non-sonicated raw milk (a1 and c1), as well as those from sonicated milk (b1 and d1), is expected. This is because the samples underwent identical treatments, and only the progression of fermentation during the first three months of ripening produced minor differences in their microbiomes. The most distinct microbiome was found in sample e1, which exhibited the highest species diversity compared to the other samples. The high microbial diversity of e1 might due to the absence of ultrasonic treatment of the raw milk at the start of cheese production, which allowed the intrinsic microbiota of milk to remain intact. Consequently, with a broader range of microorganisms present and sufficient time for growth during six months of fermentation and ripening, a large microbial community developed in this sample. Other comparative patterns, similarity and dissimilarity matrix, follow trends similar to those observed in the genetic heat maps. The presence of certain species from the Enterobacteriaceae family in the samples may result from sheep grazing on fields contaminated with wastewater and animal manure, leading to their transfer into the milk (Hrenovic & Ivankovic, 2009). The microbial load introduced during cheese production, originating from added salt, utensils, and the hands of individuals involved in cheese

handling, also contributes to the microbial diversity of the final product. Thus, in addition to lactic acid bacteria (LAB), the presence of *Acinetobacter* and *Staphylococcus* was confirmed. The occurrence of various members of the Acinetobacteraceae family (Hrenovic & Ivankovic, 2009) and *Staphylococcus* in saline environments such as cheese, has previously been reported (Jørgensen, Mørk, & Rørvik, 2005).

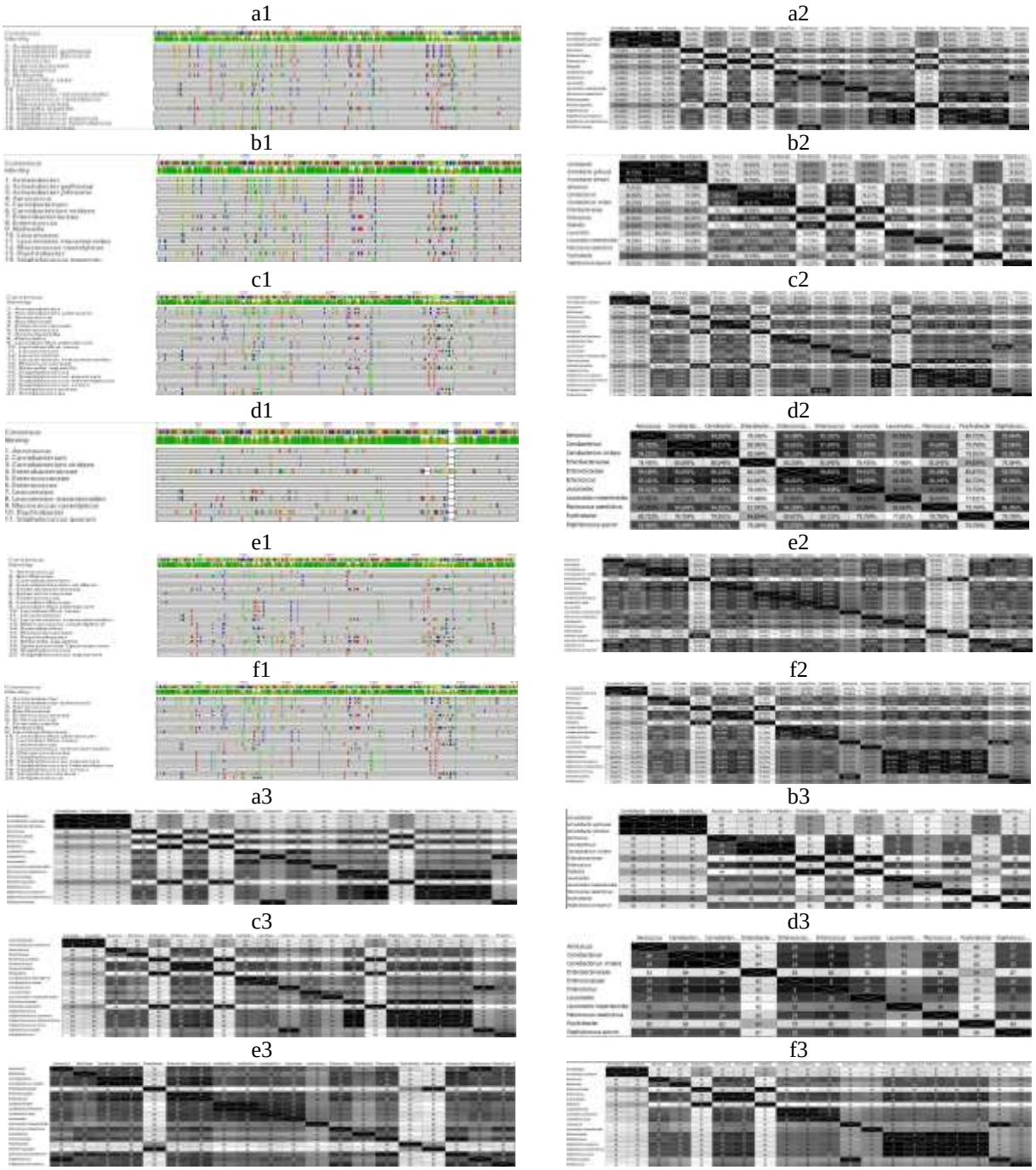


Fig. 1. Genetic heatmap (a1-f1), %identity (a2-f2) and differences matrix (a3-f3) of nucleotide sequence for cheese samples (a-f)

White, lowest %identity; black, highest %identity. The differences showed with the color intensity rate of each cell. Black, lowest difference; white, highest difference.

Pathways Enrichment of FAs

As shown in Fig. 2 (a), the network provides insight into the lipolysis process during siahmazgi cheese ripening and microbe-driven FA transformation. In addition, correlations between dominant species, genera and families, and FAs in cheese samples are shown in Fig. 2 (b1-b3). Based on Fig. 2 (a, b1–b3), all or some of the species and genera associated with these FAs are present in all samples of Siahmazgi cheese and may subsequently be involved in all metabolic pathways associated with the lipids presented in the network, thereby influencing the subsequent development of flavor of Siahmazgi cheese. Metabolomics enrichment (Fig. 2a) of the FA profile produced in Siahmazgi cheese samples indicated that FAs produced by the microbiome through different lipid metabolic pathways are involved. Based on this network, *S. equorum*, *A. johnsonii*, *L. zeae*, *M. caseolyticus*, *L. mesenteroides*, and *L. plantarum* were identified as key players, while hexadecanoic acid, octadecanoic acid, (9Z,12Z,15Z)-octadecatrienoic acid, and (9Z)-hexadecenoic acid were identified as the main FAs in lipid metabolism pathways. *Lactobacillus* spp., *Leuconostoc* spp., and *Staphylococcus* spp. were identified as releasing (lipases) or transforming (isomerases, hydratases, β-oxidation enzymes) FAs during ripening (Collins, McSweeney, & Wilkinson, 2003). The release of free FFAs (palmitic, stearic, oleic, and linoleic acids as flavour precursors) occurs due to lipolysis attributed by endogenous milk lipase, microbial lipases or esterases (Collins *et al.*, 2003). Conjugated linoleic acid (CLA) is a bioactive fatty acid with recognized health-promoting properties, and its production during dairy fermentation has been widely attributed to lactic acid bacteria (LAB) (Sosa-Castañeda *et al.*, 2015). Several LAB species, particularly *Lactobacillus plantarum*, are capable of converting linoleic acid into CLA or related derivatives through isomerization, hydration, dehydration, or isomerase-mediated reactions (Liu *et al.*, 2011). In addition to LAB, cheese surface microbiota such as *Macrococcus* and *Staphylococcus* play an important role in lipid metabolism, as they possess lipases and other enzymes that generate volatile and non-volatile fatty acid (FA) derivatives, including short-chain free fatty acids, methyl ketones, and esters that contribute to flavor development in hard cheese (Mazhar, Kilcawley, Hill, & McAuliffe, 2020). Environmental Gram-negative bacteria, such as *Acinetobacter*, may also be involved in long-chain FA catabolism through β-oxidation and related degradation pathways, consistent with their association with FA elongation and degradation processes (Ren & Palmer, 2023). Ultrasonic treatment of milk prior to cheese manufacture can influence lipid metabolism by modifying the indigenous microbiota through selective cell damage or inactivation, releasing intracellular enzymes such as lipases, and altering milk fat globule structure, which increases triglyceride exposure to enzymatic hydrolysis. These changes can affect both the quantity of free fatty acids produced and the microbial taxa responsible for FA metabolism (Hickey, Kilcawley, Beresford, & Wilkinson, 2007). Accordingly, the present study examined the effects of ultrasonic treatment (0, and 10 minute) on FA metabolic patterns and the microbial species associated with these processes. In control samples, dominant taxa such as *Leuconostoc mesenteroides* and *Staphylococcus equorum* were correlated with key long-chain fatty acids, including linoleic, α-linolenic, hexadecanoic, and octadecanoic acids, which are central metabolites in unsaturated FA biosynthesis, FA elongation, and degradation pathways. Across all samples, some genera such as *Lactobacillus*, *Leuconostoc*, *Macrococcus*, *Staphylococcus*, and *Acinetobacter* were associated with linoleic acid metabolism and potential CLA formation. Similar distribution patterns between microbiome-based functional predictions and measured FA profiles, together

with Procrustes analysis, demonstrated a moderate but significant concordance between microbial community structure and FA metabolism during ripening, supporting a functional link between cheese microbiota and lipid transformation processes.

Microscopic Changes in Texture of **Siahmazgi** Cheese Samples

According to Table 1 and Fig. 3, the effects of ultrasonication for 0, 5, and 10 minutes on the microscopic structure of cheese showed substantial changes in both porosity and pore number.

Table 1- Porosity analysis of Siahmazgi cheese samples in the 6 months of ripening by Image J

Sample	Porosity (0-10)	Pores detected	Magnification
Control (E)	0.008560 ± 0.0007^{c}	278± 10^{d}	1000x
	0.008243 ± 0.0009^{d}	676± 12^{c}	5000x
Ultrasound 5 min (EF)	0.046352 ± 0.0034^{a}	2244 ± 37^{b}	1000x
	0.028852 ± 0.0021^{b}	2345 ± 24^{a}	5000x
Ultrasound 10 min (F)	0.003073± 0.0013^{f}	115± 5^{e}	1000x
	0.005694 ± 0.0009^{e}	294± 8^{d}	5000x

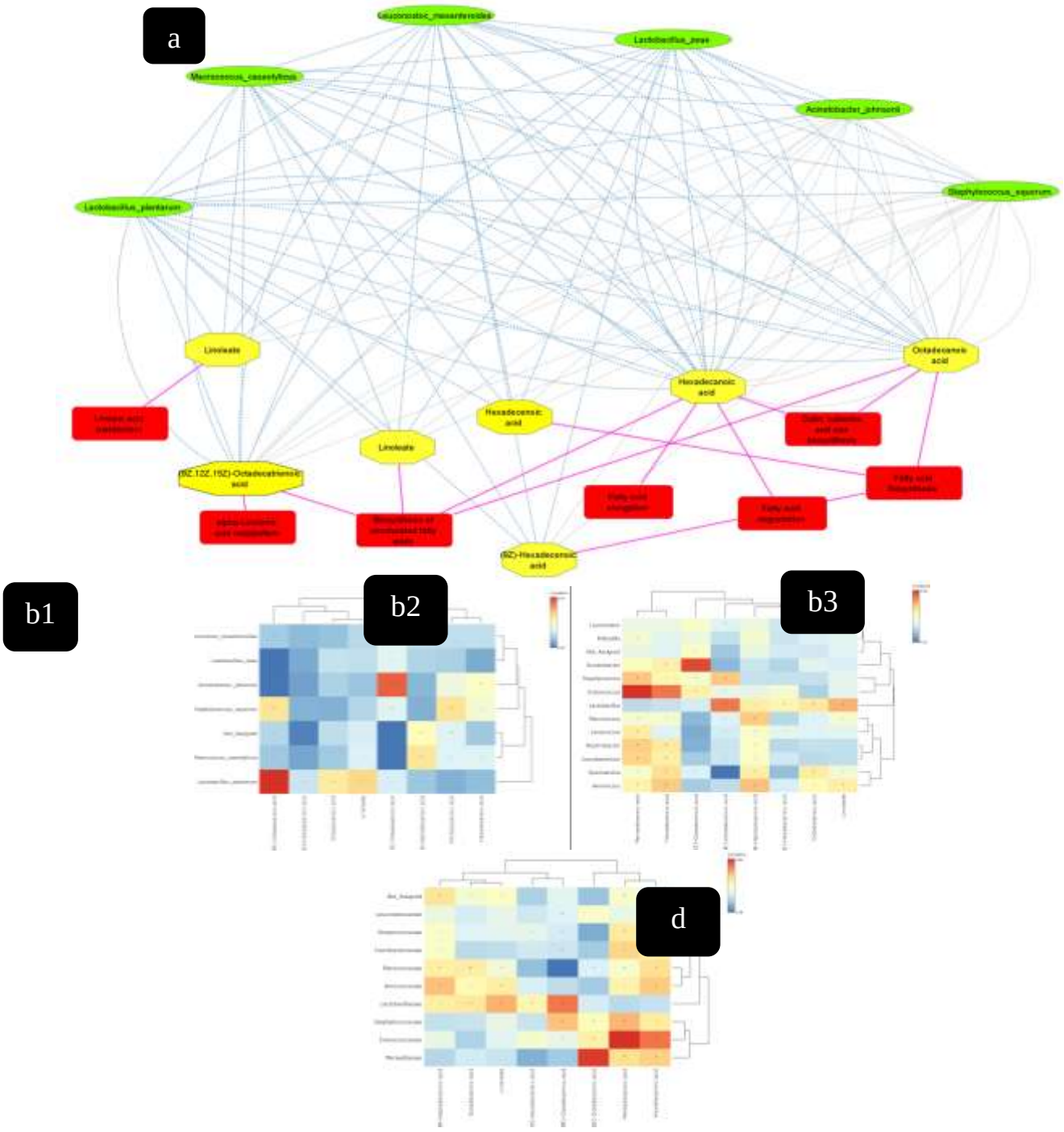
Different letters in each column indicate significant differences among the samples ($p < 0.05$).

Based on SEM image analysis, the control sample showed very low porosity values (0.0085–0.0082%) and a limited number of pores (278–676). However, 5 minutes of ultrasonication resulted in a remarkable 5- to 6-fold increase in both pore number (2244–2345) and porosity (0.04635–0.046352) compared with the control.

Ultrasonication affects cheese microstructure and fermentation primarily through cavitation, which disrupts protein interactions, opens the casein network, and creates microvoids that alter matrix porosity. Cavitation and sonoporation transiently increase cell membrane permeability, enhancing nutrient and metabolite transfer, stimulating microbial activity, and accelerating biochemical reactions. Short-term or low-intensity ultrasound generally promotes microbial growth and metabolic performance, whereas prolonged treatment can damage membranes, cause intracellular leakage, and inactivate cells, with species-specific sensitivity (*E. aerogenes* and *B. subtilis* being more susceptible than *Staphylococcus* spp. (Carrillo-Lopez *et al.*, 2021). Low-frequency ultrasound (20–30 kHz) has been shown to enhance β-galactosidase release, lactose hydrolysis, organic acid production, proteolysis, lipolysis, and bioactive compounds formation. Short-term ultrasonication increases porosity in cheese and protein gels by partially disaggregating protein networks and creating microcavities, due to physical (microstreaming, shear stress, membrane disruption) and chemical effects (free radical formation, lipid peroxidation, protein unfolding). In contrast, 10-minute treatment reduces porosity and pore number via protein reaggregation and matrix compaction (Chandrapala, Zisu, Palmer, Kentish, & Ashokkumar, 2011), whereas 5-minute ultrasonication enhances porosity through fat globule disruption and improved fat–protein distribution (Chen *et al.*, 2025). During ripening, microbial fermentation, gas production, enzyme activity, and moisture changes further modify pore structure (Yavuz, Kasavi, & Öner, 2021). Overall, short-term ultrasound produces porous cheese, while longer treatments yield denser, more uniform textures.

The structural differences observed among the nine treatments align closely with recent findings regarding the influence of ultrasound on dairy protein networks. High‑intensity ultrasound is known to induce localized cavitation, shear forces, and microstreaming, which collectively disrupt the protein matrix and promote the formation of more open and porous structure. In the present study, cheeses treated for 5 and 10 min displayed greater porosity and a less compact microstructure compared with the control, consistent with

previously reported ultrasound‐mediated loosening of protein gel networks (Teng *et al*., 2020).



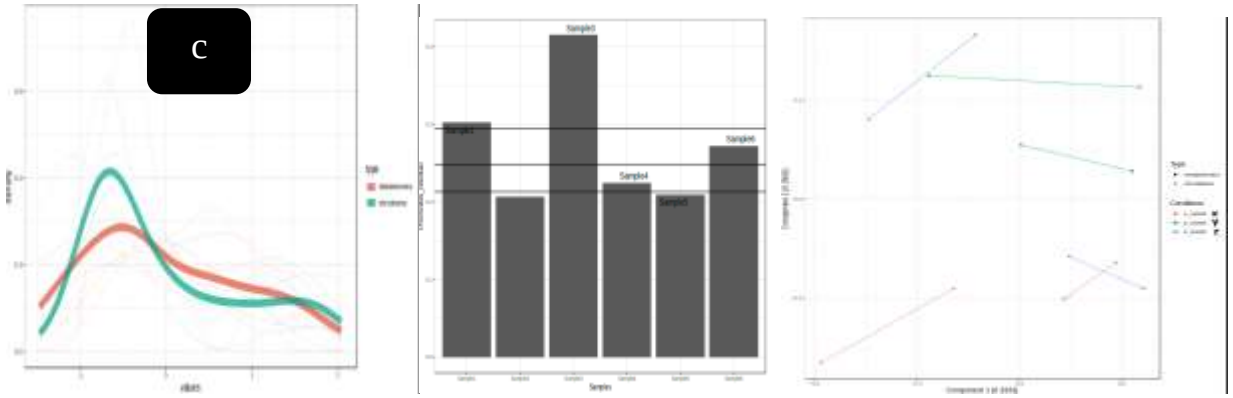


Fig. 2. The network among microbiome, FAs and lipid metabolic pathways in Siahmazgi cheese samples by Cytoscape. Rectangle: Metabolic pathway, Pentagon: FA; Elliptical: Bacteria spp., (a). The heatmap visualization of FAs produced by the dominant bacteria species (b1), genus (b2), family (b3). Density Plot **against log2 of read counts (c). Procrustes analysis, Residuals and ordination plot (d)**

The enhanced pore formation observed in the 10‑min treatment corresponds with evidence showing that prolonged exposure increases cavitational activity and modifies casein micelle aggregation. Similar microstructural patterns—characterized by expanded cavities and weakened protein junctions—have been reported in fresh cheeses exposed to high‑power ultrasound during storage (Chen *et al*., 2025). Furthermore, the quantitative porosity analysis in our study agrees with multi‑scale evaluations demonstrating that ultrasound can significantly alter cheese matrix architecture, leading to increased pore density and heterogeneity. Overall, these comparisons confirm that the structural modifications observed in Siahmazgi cheese are consistent with the broader literature describing ultrasound‑induced alterations in cheese microstructure.

Texture Profile Analysis

The results obtained from the TPA of Siahmazgi cheese samples are presented in Table 2. Hardness values showed notable changes due to both ultrasound treatment and ripening time. At 0 months, hardness increased from 8.52 ± 1.45 N in the control to 10.01 ± 0.45 N with 5-minute ultrasound and 17.79 ± 1.20 N with 10-minute treatment, reflecting immediate protein matrix compaction. By 3 months, hardness decreased in all samples, 1.36 ± 0.15 N (control), 2.13 ± 0.15 N (5 min), and 4.29 ± 0.32 N (10 min). At 6 months, hardness values were 2.11 ± 0.22 N for the control, 1.71 ± 0.22 N for 5 minutes, and 11.35 ± 0.85 N for 10 minutes.

These results indicate that the 10-minute ultrasound treatment consistently produced higher hardness, consistent with SEM observations of reduced porosity and increased structural cohesion. Adhesiveness remained negative in all treatments. At 0 months, values were −0.30 ± 0.05 N for the control, −0.28 ± 0.05 N for the 5-minute treatment, and −0.80 ± 0.10 N for the 10-minute ultrasound treatment, indicating a strong increase in surface stickiness with prolonged sonication. By 3 months, adhesiveness decreased to −0.05 ± 0.02 N (control), −0.03 ± 0.02 N (5 min), and −0.15 ± 0.03 N (10 min). At 6 months, adhesiveness increased again, with the 10-minute treatment remaining the highest at −0.50 ± 0.07 N, while the 5-minute sample showed the lowest value (−0.05 ± 0.02 N). At 0 months, cohesiveness increased slightly with ultrasound, from 0.93 ± 0.03 in the control to 0.94 ± 0.03 (5 min) and 0.96 ± 0.01 (10 min). By 3 months, cohesiveness dropped sharply in the control (0.37 ± 0.02), while the 5-minute (0.74 ± 0.05) and 10-minute (0.85 ± 0.04) treatments retained much higher structural integrity. At 6 months, cohesiveness declined in the 5-minute sample (0.57 ± 0.05), whereas the 10-minute treatment maintained a high value of 0.93 ± 0.02. Overall, ultrasound,

especially the 10-minute duration, significantly enhances and preserves casein network integrity during ripening. Springiness remained consistently high in all treatments. At 0 months, values were 0.95 ± 0.02 (control), 0.93 ± 0.01 (5 min), and 0.96 ± 0.01 (10 min), with only minor differences. After 3 months, springiness increased in the 5-minute sample (0.99 ± 0.03), exceeding the control (0.92 ± 0.01) and approaching the 10-minute treatment (0.94 ± 0.02). By 6 months, the 5-minute treatment again showed the highest springiness (0.99 ± 0.02), followed by the 10-minute (0.97 ± 0.01) and the control (0.90 ± 0.01).

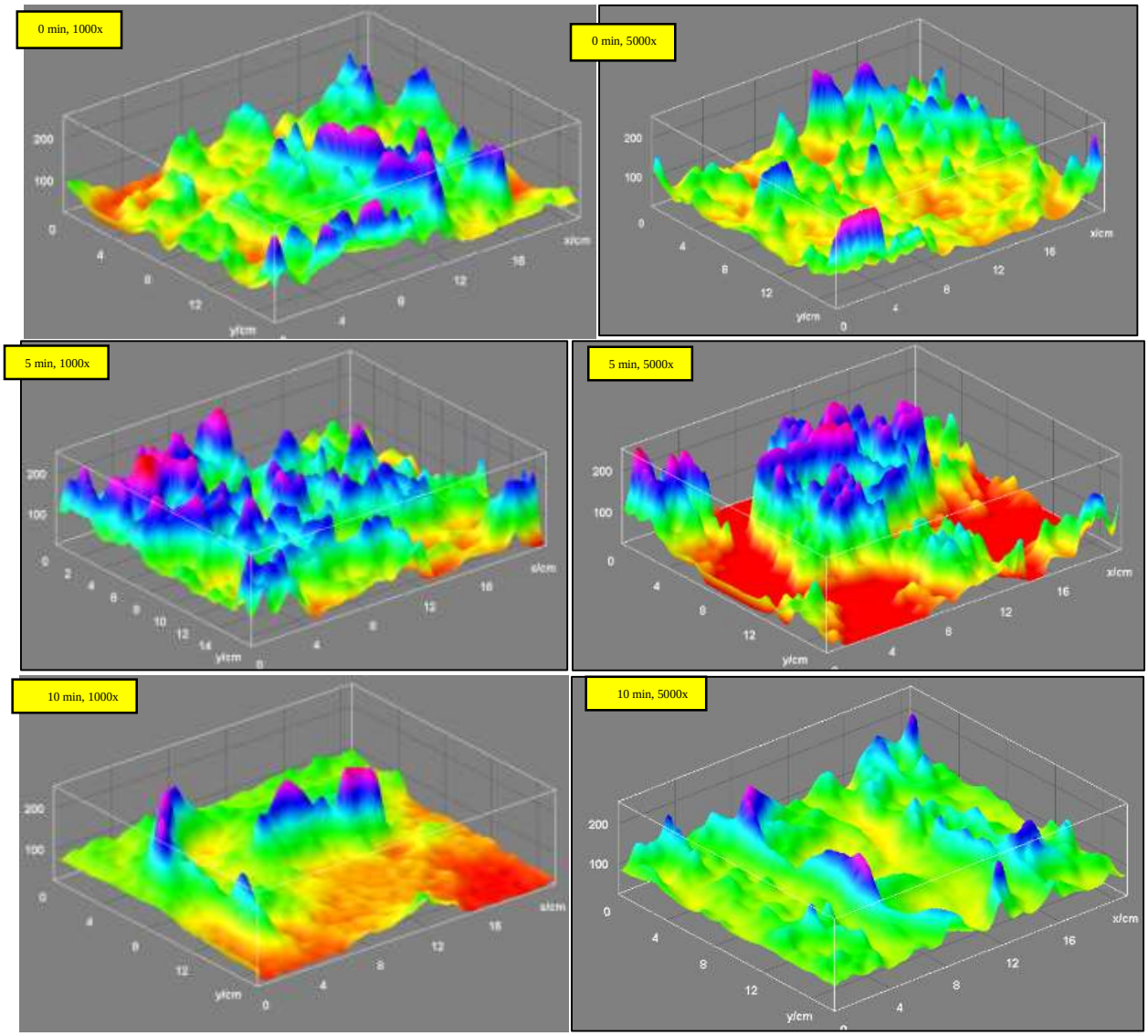


Fig. 3. The SEM of Siahmazgi cheese in the 6 months of ripening affected by 0, 5, and 10 min ultrasound

These results indicate that even short sonication enhances and maintains elasticity during ripening. At 0 months, gumminess values were 7.92 ± 0.55 N for the control, 9.23 ± 0.55 N for the 5-minute treatment, and 17.07 ± 1.15 N for the 10-minute treatment. At 3 months, gumminess was 1.57 ± 0.08 N for the 5-minute treatment, higher than the control (0.51 ± 0.08 N) but lower than the 10-minute treatment (3.65 ± 0.28 N). At 6 months, gumminess in the 5-minute sample decreased to 0.97 ± 0.18 N, below the control (1.69 ± 0.18 N). In contrast, the 10-minute treatment resulted in very high gumminess (10.50 ± 0.78

N) and a firmer, more resistant texture, indicating persistent structural integrity. At 0 months, chewiness increased from 7.52 ± 0.48 N in the control to 8.61 ± 0.48 N with 5-minute ultrasound, while the 10-minute treatment produced a much stronger effect (16.39 ± 1.08 N). By 3 months, chewiness values decreased but remained higher in sonicated samples—0.47 ± 0.07 N (control), 1.55 ± 0.07 N (5 min), and 3.43 ± 0.25 N (10 min). At 6 months, chewiness in the 5-minute sample declined to 0.96 ± 0.16 N, below the control (1.50 ± 0.16 N), whereas the 10-minute treatment maintained a very high value (10.18 ± 0.72 N). This study demonstrates that both ultrasound duration and ripening time significantly influence the texture and chewiness of Siahmazgi cheese. The effects of ultrasound followed a nonlinear pattern depending on exposure duration. At 0 months, both 5- and 10-minute ultrasound treatments increased hardness, cohesiveness, gumminess, and chewiness compared to the control, with the 10-minute treatment, the denser, most cohesive structure were achieved. Prolonged sonication enhanced protein–fat interactions, promoted reorganization of the casein network into a compact matrix, and reduced porosity, consistent with mechanisms of acoustic cavitation (Chandrapala *et al.*, 2011). This dense network remained stable during six months of ripening, resulting in a texture comparable to semi-hard cheeses, whereas the 5-minute treatment provided moderate reinforcement, suitable for soft to semi-soft textures. During the first three months, softening of all cheeses happened due to proteolysis, but ultrasound appeared to moderate the softening trend by synergistically interacting with microbial and enzymatic activity. Enhanced proteolysis in sonicated samples likely reflects increased enzyme accessibility and stimulation of lactic acid bacteria (LAB), supporting controlled softening without excessive structural degradation. By six months, moisture loss and reduced proteolytic activity contributed to matrix firming and further structural stability (Lucey, Johnson, & Horne, 2003). The control exhibited extensive softening and low cohesiveness due to uncontrolled proteolysis, while ultrasound treatments enhanced matrix uniformity, fat globule disruption, and protein–fat bonding, consistent with previous reports in Siahmazgi and other cheeses like Cheddar and Gouda (Bermúdez- Aguirre, Mawson, & Barbosa- Cánovas, 2008; Jalilzadeh, Hesari, Peighambardoust, & Javidipour, 2018). Differences in response among cheeses are attributed to inherent variations in casein matrix density, fat distribution, and microbial activity. Overall, ultrasound effectively modulated Siahmazgi cheese texture, with 10-minute treatment producing a firm, cohesive, and resilient matrix suitable for semi-hard cheese varieties, while 5-minute sonication provided moderate softening, demonstrating the method's potential for tailoring cheese textural properties during ripening.

Table 2- Effect of ultrasound treatment and ripening periods on the TPA of the cheese samples

Formulation	Hardness (N)	Adhesiveness (N)	Cohesiveness	%Springiness	Gumminess (N)	Chewiness (N)
A	8.52 ± 1.45^{c}	-0.3 ± 0.05^{d}	0.93 ± 0.03^{a}	0.95 ± 0.02^{a}	7.92 ± 0.55^{c}	7.52 ± 0.48^{c}
AB	10.01 ± 0.45^{c}	-0.28 ± 0.05^{a}	0.94 ± 0.03^{d}	0.93 ± 0.01^{a}	9.23 ± 0.55^{c}	8.61 ± 0.48^{c}
B	17.79 ± 1.20^{a}	-0.80 ± 0.1^{e}	0.96 ± 0.01^{a}	0.96 ± 0.01^{a}	17.07 ± 1.15^{a}	16.39 ± 1.08^{a}
C	1.36 ± 0.15^{f}	-0.05 ± 0.02^{a}	0.37 ± 0.02^{e}	0.92 ± 0.01^{b}	0.51 ± 0.08^{f}	0.47 ± 0.07^{g}
CD	2.13 ± 0.15^{e}	-0.03 ± 0.02^{a}	0.74 ± 0.05^{c}	0.99 ± 0.03^{a}	1.57 ± 0.08^{f}	1.55 ± 0.07^{e}
D	4.29 ± 0.32^{d}	-0.15 ± 0.03^{b}	0.85 ± 0.04^{b}	0.94 ± 0.02^{a}	3.65 ± 0.28^{d}	3.43 ± 0.25^{d}
E	2.11 ± 0.22^{e}	-0.08 ± 0.02^{a}	$0.80 \pm 0.05b^{c}$	0.90 ± 0.01^{b}	1.69 ± 0.18^{e}	1.50 ± 0.16^{e}
EF	1.71 ± 0.22^{e}	-0.05 ± 0.02^{a}	0.57 ± 0.05^{d}	0.99 ± 0.20^{a}	0.97 ± 0.18^{e}	0.96 ± 0.16^{f}
F	11.35 ± 0.85^{b}	-0.50 ± 0.07^{c}	0.93 ± 0.02^{a}	0.97 ± 0.01^{a}	10.50 ± 0.78^{b}	10.18 ± 0.72^{b}

Different letters in each column indicate significant differences among the samples ($p < 0.05$).

Conclusion

The results of this study demonstrated that ultrasound treatment significantly influenced the physicochemical, microbiological, and structural characteristics of Siahmazgi cheese during ripening. Compared with the control samples, ultrasonic treatment cheeses (5 and 10 min) showed notable modifications in microstructure, including increased porosity and pore number as revealed by SEM image analysis. These structural changes were accompanied by measurable variations in texture profile parameters throughout the ripening period. In addition, ultrasound treatment contributed to a reduction in pathogenic microorganisms compared with the untreated control samples, indicating its potential as a supportive non‑thermal processing technique to improve microbial safety in traditional cheeses. Overall, moderate ultrasound treatment, particularly at 5 min, produced favorable structural and quality changes while maintaining desirable textural characteristics of the cheese. Therefore, ultrasound processing can be considered a promising technology for improving the quality and safety of traditional Siahmazgi cheese. However, this study had some limitations. The investigation was conducted at a laboratory scale with limited ultrasound treatment conditions and a relatively small number of samples. In addition, sensory evaluation and detailed biochemical analyses of flavor compounds were not included. Further studies are recommended to evaluate a wider range of ultrasound intensities and treatment durations, investigate the long‑term effects on flavor development and consumer acceptance, and assess the scalability of this technology for industrial cheese production.

Author Contributions

Somayehalsadat Mehrzad: conducted the experiments, collected and processed the data, and prepared a draft of the manuscript. **Fakhri Shahidi and Nafiseh Davati**: supervised and designed the experiments, interpreted the data, contributed to the writing of the original draft, and revised the manuscript. **Mostafa Karami**: co-supervisor on this project; provided general guidance throughout the development of the work.

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مقاله پژوهشی
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تأثیر فراصوت بر تحلیل مشخصات بافت، فیلوژنی میکروبیوم و پیش‌بینی مسیرهای متابولیکی اسیدهای چرب در پنیر سیاه‌مزگی

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چکیده

پنیر سیاه‌مزگی از طریق فعالیت‌های متابولیکی میکروبیوتای بومی موجود در شیر پاستوریزه‌نشده دچار رسیدگی می‌شود. برای تضمین ایمنی این محصول، به‌کارگیری روش‌های فرآوری غیرحرارتی مانند فراصوت ضروری است. هدف این مطالعه بررسی تغییرات بافتی و روابط فیلوژنتیکی میکروبیوم پنیر سیاه‌مزگی تحت تأثیر تیمار فراصوت و به‌دنبال آن انجام تحلیل متابولومیک اسیدهای چرب بود. تغییرات میکروسکوپی بافت پنیر تحت تیمارهای فراصوت (۰، ۵ و ۱۰ دقیقه) از طریق پردازش تصاویر SEM با استفاده از نرم‌افزار ImageJ ارزیابی شد. همچنین تحلیل پروفیل بافت با دستگاه سنجش بافت انجام گرفت. تحلیل‌های فیلوژنتیکی و متابولومیکی میکروبیوم با استفاده از نرم‌افزار Geneious Prime و بررسی ارتباطات بین میکروب‌ها، متابولیت‌ها و مسیرهای متابولیکی با استفاده از Cytoscape انجام شد. نتایج تحلیل فیلوژنتیکی نشان داد میکروبیوم پنیرهای تیمارشده با فراصوت مشابه (از نظر مدت زمان) تا ماه سوم رسیدگی، الگوهای حرارتی ژنتیکی مشابهی داشتند، در حالی‌که نمونه بدون تیمار در ماه ششم رسیدگی بیشترین تنوع زیستی را نشان داد. گونه‌های *Staphylococcus equorum*، *Acinetobacter johnsonii*، *Lactobacillus zeae*، *Macrococcus caseolyticus*، *Leuconostoc mesenteroides* و *Lactiplantibacillus plantarum* به‌عنوان عوامل کلیدی دخیل در متابولیسم اسیدهای چرب شناسایی شدند. هگزادکانوئیک اسید، اکتادکانوئیک اسید، (9Z,12Z,15Z)-اُکتادکاترینوئیک اسید و (9Z)-هگزادسنوئیک اسید نیز به‌عنوان اسیدهای چرب اصلی حاصل از متابولیسم لیپیدها و زیست‌سنتز اسیدهای چرب غیراشباع شناسایی شدند. نمونه‌های تیمارشده با ۵ دقیقه فراصوت بیشترین تعداد حفره (۲۳۴۵ عدد) و تخلخل (۰/۰۴۶۳۵۲) را داشتند. در طول دوره رسیدگی، سختی، چسبندگی، انسجام، صمغی‌بودن و قابلیت جویدن در نمونه‌های تیمار ۵ دقیقه‌ای کمترین و در نمونه‌های تیمار ۱۰ دقیقه‌ای بیشترین مقدار را نشان دادند. بنابراین، در صورتی‌که هدف دستیابی به بافتی نرم و متخلخل در پنیر سیاه‌مزگی باشد، تیمار فراصوت به‌مدت ۵ دقیقه توصیه می‌شود. افزون بر این، نتایج این پژوهش درک جامعی از تأثیر فراصوت بر ویژگی‌های بافتی، ساختار میکروبی و پیش‌بینی مسیرهای متابولیکی اسیدهای چرب در پنیر سیاه‌مزگی ارائه می‌کند که می‌تواند در توسعه فناوری‌های غیرحرارتی برای پنیرهای سنتی مؤثر باشد.

واژه‌های کلیدی: پنیر سیاه‌مزگی، تحلیل پروفیل بافت، فراصوت، متابولومیکس

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