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A Comparative Study on Yayık Butter Produced with Commercial and Endemic Yogurt Starter Culture Strains

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ABSTRACT
Starter cultures are involved in biochemical reactions during fermentation resulting in the diversity of foods. They are also used for aroma/flavor, texture, nutritional quality enhancement, and shelf-life extension. This study aimed to evaluate the effects of using four different starter culture combinations of which two were isolated starter cultures of *Streptococcus thermophilus* (St) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Lb) and two were commercial cultures of the same bacteria in Yayık butter characteristics. For this purpose, four different yogurt samples were prepared and then churned to obtain four different Yayık butter samples. Physico-chemical analysis, free fatty acids, volatile compounds, and sensory analysis were performed on the 1st, 30th, and 60th days of storage. No significant differences were observed in moisture, fat, titratable acidity, acid degree, and peroxide value between the samples (P>0.05). However, endemic isolated combined cultures showed better performance in terms of free fatty acid formation and sensorial attributes. The serum pH differed significantly among the samples with higher values in isolated cultures (P<0.05). Storage time was significantly effective on the titratable acidity (°SH) of all Yayık butter samples (P<0.01). The peroxide values were lower than the threshold value of 2.0 meq O_2/kg fat. Acid degree varied between 1.70-1.75 mg KOH/g during the storage period. Endemic isolated cultures exhibited the highest free fatty acid accumulation. In the Yayık butter samples, a total of 31 volatile compounds were quantified. The highest number was detected in the butter samples produced with isolated strains (27St/27Lb and 27St/ALb). Yayık butter samples produced with 27St/27Lb contained 16, while with 27St/ALb had 7 compounds identified. Butyric acid and hexanoic acid were the most abundant carboxylic acids while ketones were the predominant volatile compound detected in all Yayık butter samples. This study highlights the importance of preserving traditional culture strains and offers another perspective on using them in dairy industry.

Keywords: Yayık butter, Isolated strains, Lipolysis, Oxidative stability, Volatile compounds, Sensory analysis

1. Introduction

Butter is one of the essential products of the dairy industry known for its sensory attributes and nutritional value (Anonymous 2005; Üçüncü 2005). The production of butter remains significant in the Turkish dairy market, with over 70,000 tonnes being produced (TÜİK 2023). Among the butter types, Yayık butter is a traditional dairy product originating from yogurt and differs from cream butter in terms of raw material. Yayık butter is made from a concentrated form of the milk fat found in yogurt. The yogurt is produced 12-48 hours before churning and serves as the raw material of Yayık butter. This unique process contributes to specific flavor-aroma characteristics that set Yayık butter apart from butter made from cream (Atamer et al. 2004; Atamer et al. 2005). Yayık butter is primarily produced in the northwestern regions of Türkiye, including the Black Sea, and Southern and central Anatolia (Atamer et al. 2006). The production of this butter is typically carried out on a family scale and sold in local markets (Şenel et al. 2010). Yayık butter combines the health benefits and unique organoleptic properties of yogurt with the long shelf-life advantage provided by fermentation.

In recent years, consumer demand for fermented dairy products has grown significantly due to their health-enhancing capacity. Lactic acid bacteria (LAB) are used as starter cultures in the production of dairy products such as yogurt, cheese, and butter. They play a crucial role in enhancing some characteristics such as flavor, texture, and nutritional value (Kleerebezem & Hugenholtz 2003; van Hylckama & Hugenholtz 2007). LAB can synthesize acetic acid, acetaldehyde, ethanol, diacetyl, and alcohol that contribute to the flavor and texture of fermented products. This is primarily due to lactic acid metabolism, which is the main component in fermentation processes (van Hylckama & Hugenholtz 2007; Caplice & Fitzgerald 1999). LAB have been employed in food development for their therapeutic properties and preservative effects since ancient times (Chittora et al. 2022). They serve multiple roles, such as acting as starter cultures in food fermentation, adjunct cultures that enhance taste, texture and nutritional quality, and as bio-protective agents against spoilage and pathogenic bacteria. Additionally, they contribute to texture improvement through the production of exopolysaccharides and play various roles in medicine and agriculture (Maiouet et al.

2024). Traditional production practices applied in fermented dairy products supply diversity in the culture environment and affect the organoleptic properties of these products. These variations ultimately influence the shelf life of the final product, as they are impacted by variations in pH, the environment, the fermenting population, fermentation conditions, etc. (Aleksanyan et al. 2024).

In yogurt fermentation, LAB such as *Streptococcus thermophilus (*St) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Lb) are used in a 1:1 combination ratio and are responsible for the aroma/flavor of yogurt and Yayık butter (Sağdıç et al. 2004). The back-slopping fermentation used in traditional yogurt making involves transferring a small amount of previously fermented yogurt into the fresh, heat-treated milk. In commercial applications, commercial starter cultures are used in yogurt production (Wirawati et al. 2019). The back-slopping enhances both microbiological diversity and the sensory attributes of yogurt. While using commercial cultures can lead to standardization in processing, it may result in some loss of the characteristic quality criteria of yogurt (Uzunsoy et al. 2021).

Numerous studies have been conducted on Yayık butter; covering various aspects such as its general composition, and microbiological properties. In one of the previous researches on Yayık butter; Hayaloglu and Konar (2001) focused on Yayık butter samples sold in local markets. Alwazeer et al. (2024) examined the impact of using hydrogen, nitrogen, and synthetic antioxidants (BHT) on some characteristics of Yayık butter samples. While some studies investigated the use of commercial starter culture in production (Sağdıç et al. 2004; Gundogdu et al. 2020), some others examined the potential production technologies of Yayık butter at the laboratory scale (Atamer et al. 2005; Şenel 2006; Atamer et al. 2007; Öztekin Öztürk 2010; Şenel et al. 2010). In a study conducted by Sağdıç et al. (2002), the researchers isolated and identified LAB from Yayık butter samples collected from different locations of Türkiye. They also determined the physico-chemical and sensory properties of cream butter samples produced using these isolated LAB strains. As reported by Şenel & Atamer (2015), starter cultures used in yogurt making are key factors influencing the Yayık butter quality. However, a review of the literature reveals that there have been no studies investigating the use of traditionally produced yogurt-originated isolated endemic strains of LAB in Yayık butter production.

The conducted study is a comparative analysis focusing on the investigation of quality attributes of Yayık butter samples produced using two different isolated strain combinations and two commercial yogurt starter cultures. The samples were analyzed according to their chemical and sensorial properties, volatile compounds, and storage stability during a 60-day storage period. The study aims to investigate the use of isolated strains of yogurt bacteria obtained from local yogurt samples in Turkish regions for the production of Yayık butter. Moreover, it compares the attributes of Yayık butter samples produced by commercial culture used. Given the diversity of local dairy products in our region; it is important to reveal the effects of isolated strains on product characteristics, for both potential culture preservation and industrial applications. The results obtained from this study could serve as a base for further research in this area and for exploring the use of LAB in the dairy industry.

2. Material and Methods

2.1. Material

The raw cow's milk was obtained from the Dairy Plant of Ankara University Faculty of Agriculture (Ankara, Türkiye) and the raw cow's milk cream used for standardizing the fat content of milk up to 7%, was obtained from Atatürk Orman Ciftligi Dairy Plant (Ankara, Türkiye). Details about the samples and the design of the study are given in Table 1. The isolated strains used in the study were *Streptococcus thermophilus* (27St) and *Lactobacillus delbrueckii* subsp. *bulgaricus* (27Lb), which belonged to the culture collection of Dairy Technology Department of Ankara University Faculty of Agriculture (Uzunsoy et al. 2018). While the other isolated *Lactobacillus delbrueckii* subsp. *bulgaricus* (ALb) strain was obtained from the starter culture collection of the Food Engineering Department of İnönü University (Malatya, Türkiye). The other two starter cultures used in the study were commercial starter cultures. The culture CH-1 (Chr. Hansen Bøge Allé 10-12 DK-2970 Hørsholm, Denmark) and Lyofast Y 080 F (Sacco, Italy) were obtained from the Dairy Plant of Ankara University Faculty of Agriculture, Ankara, Türkiye, and Milkaş Gıda San., and Tic. LLC. Company (İstanbul, Türkiye), respectively.

Table 1- Design of Yayık butter production

Sample Code		***Starter culture***	
Yayık Butter	***Yogurt***	***Isolated strains***	***Commercial cultures***
A	YA	27St / 27Lb	-
B	YB	27St / ALb	-
C	YC	-	CH1
D	YD	-	Y080

Sample codes denote; A: Yayık butter produced by yogurt (YA) inoculated by isolated strains of 27St/27Lb culture combination; B: Yayık butter produced by yogurt (YB) inoculated by isolated strains of 27St/ALb culture combination; C: Yayık butter produced by yogurt (YC) inoculated by commercial culture of CH1; D: Yayık butter produced by yogurt (YD) inoculated by commercial culture of Y080; Isolated strains: 27St: denotes the isolated strain of *Streptococcus thermophilus,* 27Lb: denotes the isolated strain of *Lactobacillus delbrueckii* subsp. *bulgaricus,* ALb: denotes the isolated strain of *Lactobacillus delbrueckii* subsp. *bulgaricus,* Commercial cultures: CH1: denotes the commercial starter culture of CH-1, Y080: denotes the commercial starter culture of Lyofast Y080 F

2.2. Culture preparation and yogurt making

Each isolated strain (20 µL) was initially pre-activated in 5 mL of MRS broth (Merck, Darmstadt, Germany) for *Lactobacillus delbrueckii* subsp. *bulgaricus* and in 5 mL of M17 Broth (Merck, Darmstadt, Germany) for *Streptococcus thermophilus*. Incubation was followed at 37 °C for 72h under anaerobic conditions and at 37 °C for 24h under aerobic conditions for *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*, respectively.

From each pre-activated culture (approximately 7 log CFU/ mL each), 0.5 mL was transferred to 50 mL of sterile reconstituted milk (12% w/v) for preparing combined yogurt starter cultures in 1:1 ratio and incubated at 43 °C until complete gelation. After that serial transfer was done from 50 mL bulk culture to the required 500 mL culture for yogurt making and again incubated at similar conditions. The commercial starter cultures were also activated in 500 mL of sterile reconstituted milk (12% w/v) at 43 °C until gelation. The active cultures were stored at 4 °C overnight before yogurt production.

The design of the study is given in Table 1 and starter culture activation, yogurt preparation, and Yayık butter production stages are given in Figure 1 as a flow diagram. In the production of Yayık Butter, the first step was the activation of both the isolated strains and the commercial strains used in yogurt making. For yogurt preparation which served as the raw material of Yayık butter (Table 1), the milk was initially divided into four equal parts and inoculated by four different yogurt starter cultures prepared in the first step and in the production of yogurt samples (YA, YB, YC, YD) and the Yayık butter samples (A, B, C, D) the flow diagram (Figure 1) was followed.

2.3. Yayık butter production

In the production of Yayık butter, the yogurt samples (YA, YB, YC, YD) prepared the day before churning were kept refrigerated overnight at 4±1 °C. For each Yayık butter sample (A, B, C, D) 15 kg of yogurt was prepared. The study was conducted in triplicate. Yayık butter production was followed according to the method proposed by Atamer et al. (2007) and given in Figure 1. Finally, Yayık butter samples were packaged in sterile polystyrene containers and stored at 4±1 °C for 60 days. Some stages of Yayık butter production were represented by photographs in Figure 2. In the study, yogurt samples were analyzed on the 1st day of production, while Yayık butter samples were analyzed in the 1st, 30th, and 60th days of storage.

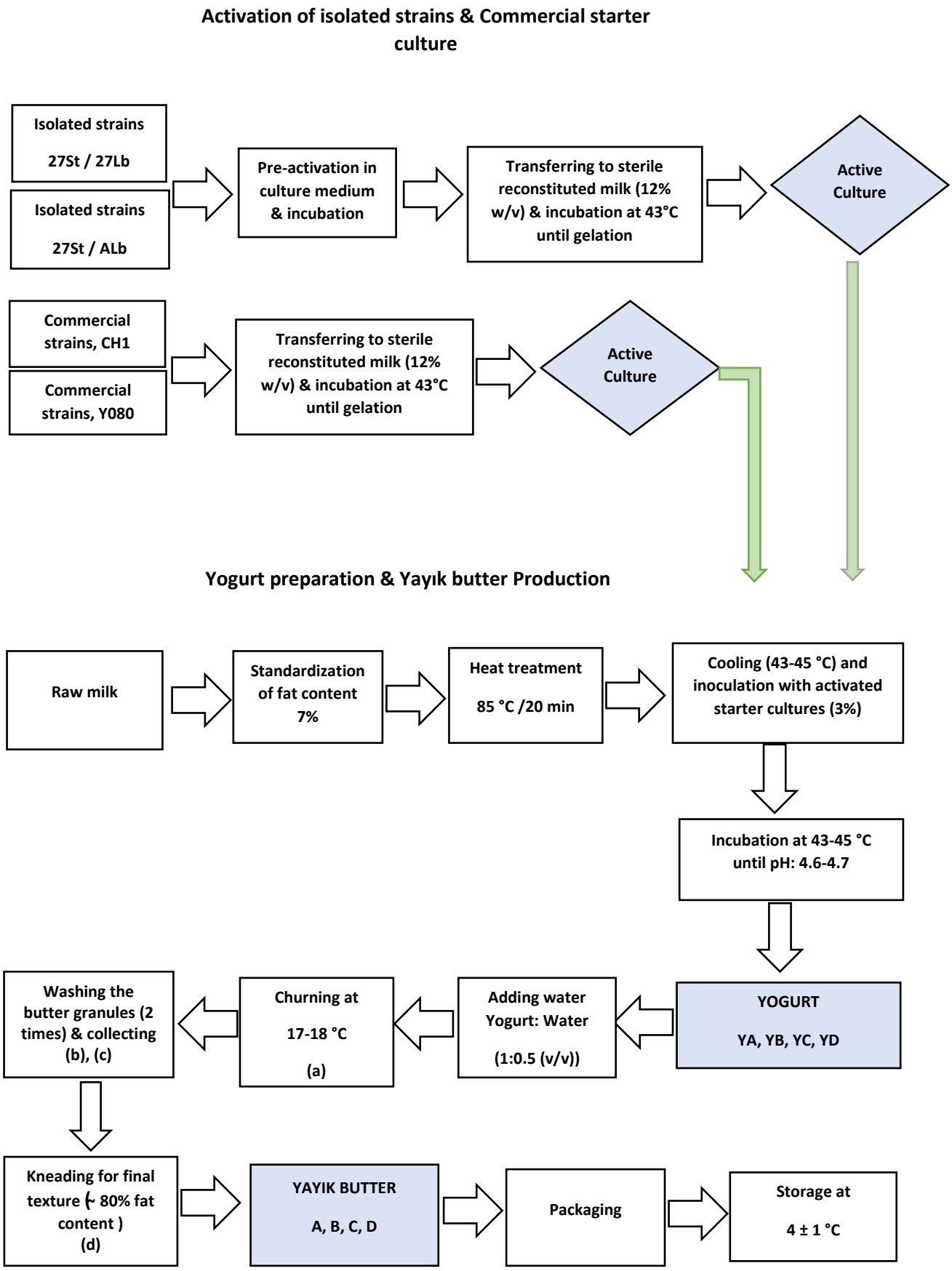

Figure 1- Flowchart of Culture Activation, Yogurt Preparation, and Yayık Butter Production Processes

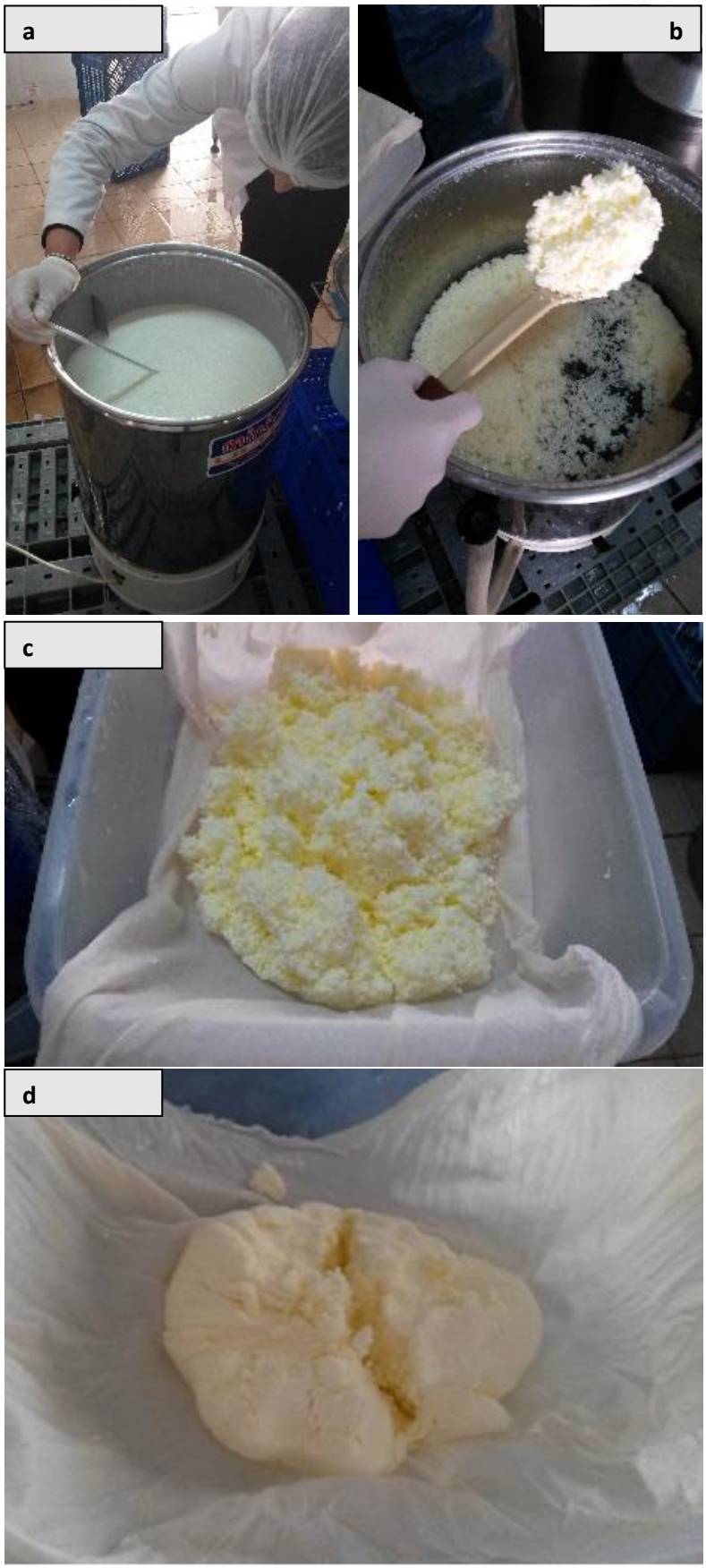

Figure 2- Yayık butter production stages by photographs
(a: Temperature control in the churning stage, b: collecting the butter granules after the washing stage, c: collected butter granules prior to kneading, d: kneading stage for reaching the final texture of butter)

2.4. Determination of chemical characteristics of yogurt and Yayık butter samples

The fat contents of both yogurt samples and Yayık butter samples were determined by the Gerber method and the dry matter contents (%) of yogurt samples and Yayık butter samples were determined using the gravimetric method (AOAC 1997). The pH values of yogurt samples and the serum pH values of Yayık butter were measured by a digital pH meter (Mettler Toledo Seven2Go S2; Schwerzenbach, Switzerland). The titratable acidity values of yogurt and Yayık butter samples were determined by titration (AOAC 1997) and calculated as Soxhlet-Henkel (˚SH). The dry matter content of the butter samples was determined by the gravimetric method (AOAC 1997), the water content of the samples was calculated by subtracting from 100.

2.5. The oxidative stability and lipolysis of Yayık butter samples

The oxidative stability and degree of lipolysis of Yayık butter samples were determined by the methods peroxide value (meq O_2/kg fat) and acid degree (mg KOH/g fat) of Downey (1975), respectively. The peroxide values of the samples were calculated by Eq. 1 and the acid degree values of the samples were calculated by Eq.2. For the analysis, fat extraction from Yayık butter samples was followed according to the methods given in AOAC (2000).

$$Peroxide\ value\ (meq\ O_2/kg\ fat) = (\mu gFe/gfat \times 55.85) = F/(m \times 55.85) \quad \text{(Eq.1)}$$

F: Amount of μg Fe corresponding to the measured absorbance value
m: Fat analyzed in grams

$$Acid\ degree\left(\frac{mgKOH}{gfat}\right) = \frac{(Amount\ 0.1N\ KOH\ \ x\ Normality\ of\ KOH\ x\ 56.1)}{Sample\ amount\ (g)} \quad \text{(Eq. 2)}$$

2.6. Determination of free fatty acids (FFAs) by Gas Chromatography

The free fatty acid content of yogurt samples and Yayık butter samples was determined by the method of Deeth, Fitz-Gerald, and Snow (1983), and details related to the process were given by Şenel et al. (2011a and 2011b). The FFA content of yogurt samples was determined on the 1st day and for Yayık butter samples on the 1st, 30th and 60th days of storage. The sample preparation involved weighing 2.5 g of yogurt and 1.5 g of butter, followed by adding 2.5 g of Na_2SO4 for each sample. The 5 mL of internal standard (C_7) and 300 µL of H_2SO_4 were then poured, and the mixture was thoroughly vortexed for 1 minute. The 5 mL of hexane was added, and the samples were rested for 1 hour prior to extracting the liquid phase from Biorad column with deactivated alumina. Each sample was eluted twice per column. The columns were then washed twice with 5 mL of hexane/diethyl ether (1:1) and dried with 5 psi of air. The FFAs held by dried alumina were transferred to test tubes and then 2 mL of 6% formic acid in ether was added. After centrifuging at 2000g for 10 minutes, the clear portion was transferred to vials using a Pasteur pipette and samples were stored at -18 °C until performing analysis. Analysis was done by GC instrument (Agilent 6890 series, Agilent Tech. Inc., CA, USA) by the operation conditions as; Flame Ionization Detector (FID) (Agilent Tech. Inc., CA, USA) with an operating temperature of 260 °C, a polyethylene glycol capillary column with dimensions of 30 m x 320 µm (inner diameter) and a 0. 25 µm film thickness (HP-FFAP Agilent Tech. Inc., Model 19091F-433). Injection conditions were as split injection with a 1:10 split ratio, with an injection volume of 5 µL at 250 °C, flow rates for the gases were as H_2: Air: N_2 = 33:370:30 ml min^{-1}. The oven temperature program was set as; starting at 120 °C for 0 minutes, increasing to 200 °C at a rate of 10 ºC min^{-1} and holding at 200 °C for 2 minutes, increasing to 205 °C at a rate of 10 ºC min^{-1} and holding for 2 minutes, then increasing to 210 °C at a rate of 10 °C min^{-1} and holding for 2 minutes, then increasing to 215 °C at a rate of 10 °C min^{-1} and holding for 3 minutes, and finally increasing to 230 °C at a rate of 10 min^{-1} and holding for 3 minutes. In the analysis standard mix solutions were also prepared in a 6% (v/v) formic acid in ether solution and 5 µL of these solutions was injected into the GC system with the same conditions as samples injection. The chromatographic-grade fatty acid standards used in the mixed solutions were supplied by Agilent (Agilent Tech. Inc., CA, USA). The calculation of the detected FFA content of the samples was done automatically by the instrument software (HP Chenstation, USA) by selecting the internal standard calculation mode (ISTD).

2.7. Determination of volatile compounds of yogurt samples and Yayık butter samples

Volatile compounds of yogurt samples were analyzed on the 1st day of production and Yayık butter samples were analyzed on storage days of 1st, 30th and 60th by the method of Whetstine et al. (2003) using Gas Chromatography/Mass (GC/MS) instrument (Agilent 7890A GC-5975 MSD, Agilent Technologies, CA, USA). Volatile compounds of the samples were extracted by solid-phase microextraction (SPME) method. The samples were weighed in 40 mL amber vials (10 grams) and mixed with 1 g NaCl and 10µL of internal standard (2- methyl-3- heptanon and 2-methyl pentanoic acid in 81mg/kg methanol). Samples were stored at -18 °C until injection to the GC/MS instrument. Before injection samples in vials were heated and stirred at 40 °C for 30 min by Reacti Term system (Pierce Reacti-Therm I #18821) and then kept at that condition with fiber (50/30 µm Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) for absorption of volatile compounds for 30 minutes. The fiber was then injected into the capillary column of the GC/MS device. The operation conditions for GC/MS were as follows; the volatile compounds were separated using the DB Wax column; the initial temperature of the oven was 40 °C, kept for 5 minutes, then raised to 100 °C at a rate of 10 °C per minute and then increased to 200 °C at a rate of 20 °C per minutes and held

for 10 minutes. The total run time was 47 minutes. Later, the detected volatile compounds were identified in the mass spectrometer and NIST/Flavournet libraries were scanned.

In the evaluation, the peak area of volatile compounds was compared with the peak area of the internal standard Equation 3.

$$Relative\ amount\ of\ volatile\ compound\ \left(\frac{\mu g}{kg}\right) = \left[\frac{Peak\ area\ of\ volatile\ compound}{Peak\ area\ of\ internal\ standard}\right] x\ correction\ factor \quad \text{(Eq. 3)}$$

2.*8. Sensory analysis*

Sensory analysis of the Yayık butter samples was carried out by ten panelists of Dairy Technology Department Staff, experienced in sensory evaluation of dairy products, on the 1st, 30th, and 60th days of storage. Samples were coded with randomized three-digit codes and served to the panelists in plastic containers with lids, a cup of water, and a cracker for cleansing the palate. In the conducted scoring test, the panelists were asked to evaluate the samples according to taste, odor, appearance, texture, and overall acceptability by using a 9-point hedonic scale (9=like immensely, 8=like very much, 7= like moderately, 6=like slightly, 5=neither like or dislike, 4=dislike slightly, 3=dislike moderately, 2=dislike very much, 1=dislike extremely).

2.9. Statistical analysis

All the data was statistically evaluated by Minitab statistical software (version 16.0, Minitab Inc., State College, PA, USA). The significance of the difference between the means of yogurt and Yayık butter samples was determined by using Duncan's Multiple Comparison Test at significance levels of $P<0.05$ and / or $P<0.01$. The study was designed in factorial order by considering storage time as a factor with 3 levels (1st, 30th, and 60th day) and the use of four different yogurt starter culture combinations (27St/27Lb, 27St/Alb, CH1, and Y0.80) as another factor.

3. Results and Discussion

3.1. *The chemical characteristics of yogurt samples*

Some chemical characteristics of yogurt samples used as the raw material of Yayık butter samples are given in Table 2.

Table 2 - Chemical characteristics of yogurt samples

Parameter	***Yogurt samples***			
	YA	***YB***	***YC***	***YD***
Dry matter (%)	14.13 ± 0.42	13.73 ± 0.72	14.25 ± 0.15	13.86 ± 0.63
Fat (%)	6.10 ± 0.58	5.67 ± 0.40	6.23 ± 0.09	6.07 ± 0.50
Titratable acidity (°SH)	36.87 ^{ab}± 0.01	26.24 ^{b} ± 0.36	47.30 ^{a}± 1.05	41.61 ^{a} ± 0.35

Data given were the $\bar{X} \pm S_X$ of the parameters analyzed in all yogurt samples of three replicates **(n=3)** Yogurt samples: YA: Yogurt inoculated by isolated strains of 27St/27Lb culture combination; YB: Yogurt inoculated by isolated strains of 27St/ALb culture combination; YC: Yogurt inoculated by commercial culture of CH1; YD: Yogurt inoculated by commercial culture of Y080. *Within a row, the difference between group averages is statistically significant ($P<0.05$). Differences between means that share the same letter are not statistically significant.

According to Table 2, no significant difference was observed among the yogurt samples (YA, YB, YC, YD) in terms of dry matter (%) and fat (%) content due to standardization of fat content applied to the raw milk prior to yogurt-making ($P>0.05$). As it was observed in the samples (Table 2), the increase in dry matter (%) is expected to be parallel with the increase in fat (%) content. However, there was a significant difference ($P<0.05$) in the titratable acidity of yogurt samples (Table 2) possibly associated with the use of different starter cultures with different acidification capacities in yogurt making. Among the yogurt samples, YB was significantly different from YC and YD inoculated with commercial starter cultures ($P<0.05$). The yogurt YC got the highest titratable acidity value followed by YD, YA, and YB samples in order.

3.2. The physico-chemical properties of Yayık butter samples

Table 3 illustrated the changes in the physico-chemical properties of Yayık butter samples during the storage period. The effect of using different starter culture combinations and storage time on the parameters analyzed were discussed according to the statistically significance issue in the following parts.

Table 3 – Chemical, oxidative stability and lipolysis characteristics of Yayık butter samples during storage*

	Yayık butter samples											
	A			*B*			*C*			*D*		
Parameter	*Day 1*	*Day 30*	*Day 60*	*Day 1*	*Day 30*	*Day 60*	*Day 1*	*Day 30*	*Day 60*	*Day 1*	*Day 30*	*Day 60*
Moisture (%)	15.91 ± 0.41	15.30 ± 0.60	16.00 ± 0,37	16.82 ± 0.66	16.27 ± 0.46	15.94 ± 0.50	17.78 ± 0.80	16.03 ± 0.35	17.70 ± 0.91	16.54 ± 0.37	16.35 ± 0.19	16.91 ± 0.29
Fat (%)	80.92 ± 0.36	80.92 ± 0.80	80.83 ± 0.60	81.83 ± 0.88	80.08 ± 0.08	80.50 ± 0.29	79.75 ± 0.38	79.83 ± 0.88	80.00 ± 1.32	80.17 ± 0.44	80.25 ± 0.25	81.25 ± 0.00
Serum pH	4.62 ± 0.21	4.39 ± 0.35	4.47 ± 0.22	4.96± 0.05	4.80 ± 0.18	4.85 ± 0.13	4.23 ± 0.02	4.09 ± 0.13	4.12 ± 0.03	4.15 ± 0.08	4.03 ± 0.17	4.21 ± 0.07
Titratable acidity (°SH)	3.43 ± 0.16	3.97 ± 0.39	3.67 ± 0.33	3.83± 0.18	3.97 ± 0.08	3.77 ± 0.18	3.96 ± 0.87	4.32 ± 0.92	3.91 ± 0.77	3.63 ± 0.76	4.25 ± 0.18	4.13 ± 0.44
Acid degree (mg KOH/g fat)	1.74 ± 0.12	1.63 ± 0,08	1.75 ± 0.12	1.70± 0.08	1.78 ± 0,20	1.75 ± 0.15	1.68 ± 0.10	1.72 ± 0,13	1.81 ± 0.15	1.71 ± 0.12	1.60 ± 0,10	1.79 ± 0.13
Peroxide value (meq O_2/kg fat)	0.12 ± 0.07	0.23 ± 0.05	0.31 ± 0.06	0.23 ± 0.05	0.27 ± 0.02	0.25 ± 0.13	0.21 ± 0.01	0.28 ± 0.05	0.31 ± 0.03	0.22 ± 0.04	0.28 ± 0.02	0.24 ± 0.03

*: Values are the $\bar{X} \pm S_{\bar{X}}$ on storage days of 1st, 30th and 60th (n=3) with no significant difference (P>0.05). Yayık butter samples: A: Yayık butter produced by yogurt (YA) inoculated by isolated strains of 27St/27Lb culture combination; B: Yayık butter produced by yogurt (YB) inoculated by isolated strains of 27St/ALb culture combination; C: Yayık butter produced by yogurt (YC) inoculated by commercial culture of CH1; D: Yayık butter produced by yogurt (YD) inoculated by commercial culture of Y080

The data in Table 4, were given as means ± SE of values of Yayık butter samples from the 1st day to the 60th day of storage.

Table 4 - Chemical properties of Yayık butter samples during the storage period*

Parameter	*Yayık butter samples*			
	A	***B***	***C***	***D***
Moisture (%)	15.74 ± 0.26	16.34 ± 0.30	17.16 ± 0.46	16.60 ± 0.17
Fat (%)	80.89 ± 0.59	80.81 ± 0.38	79.86 ± 0.47	80.56 ± 0.23
Serum pH	4.49^{ab*} ± 0.14	4.83^{a*} ± 0.08	4.15^{b*} ± 0.04	4.13^{b*} ± 0.06
Titratable acidity (˚SH)	3.69 ± 0.17	3.86 ± 0.08	4.06 ± 0.43	4.00 ± 0.30
Acid degree (mg KOH/g fat)	1.71 ± 0.06	1.75 ± 0.08	1.74 ± 0.06	1.70 ± 0.06
Peroxide value (meq O_2/kg fat)	0.24 ± 0.03	0.25 ± 0.04	0.27 ± 0.02	0.25 ± 0.02

*: Values are the $\bar{X} \pm S_{\bar{X}}$ from 1st day to 60th day of storage (n=9). * Denotes the significance level of difference P<0.05. A: Yayık butter produced by yogurt (YA) inoculated by isolated strains of 27St/27Lb culture combination; B: Yayık butter produced by yogurt (YB) inoculated by isolated strains of 27St/ALb culture combination; C: Yayık butter produced by yogurt (YC) inoculated by commercial culture of CH1; D: Yayık butter produced by yogurt (YD) inoculated by commercial culture of Y080

The moisture content of butter samples should be lower than 16% for a successful shelf life (Anonymous 2005) and kneading is a crucial step in butter processing that influences the final moisture content. However, since the Yayık butter samples were hand-kneaded during production, minor fluctuations occurred in experimental Yayık butter samples (Table 3), but these fluctuations were not found statistically significant on storage days (P>0.05) for the samples and among the mean values of the samples during on the 1st, 30th and 60th days of storage (P>0.05) (Table 4). In terms of fat content (%), all Yayık butter samples were consistent with the legal limits of 80% fat content at least, given for legal regulations of cream butter (Anonymous 2005).

Among the parameters tested for Yayık butter samples, means ± SE of serum pH was a unique item found statistically significant among the samples during 60-day storage (P<0.05) and ranged between the values of 4.13-4.83 (Table 4). The use of different starter culture combinations (Table 1) with different acidification potential in yogurt productions (YA, YB, YC, YD) and in Yayık butter samples (A, B, C, D) was responsible for these differences, and among the used cultures, commercial cultures exhibited higher activity. The serum pH of sample B got the highest mean values (4.83 ± 0.08) during the storage period and was significantly different than C and D (P<0.05). In literature, researchers have obtained varying results regarding serum pH (Sağdiç et al. 2002; Şenel et al. 2011b; Gundogdu et al. 2020). However, the Yayık butter samples were not statistically different than each other during 60-day storage in terms of titratable acidity (means ± SE) (P>0.05) (Table 4). According to Table 2, titratable acidity values of yogurt samples (YA, YB, YC, YD) indicated that YA and YB had less acidic character than YC and

YD. This is likely because the commercial cultures used in YC and YD had superior acidification capacity compared to the isolated strains used in YA and YB. Similar results were observed for the serum pH of samples C and D with lower pH and higher titratable acidity values than samples A and B (Table 4). Means of titratable acidity values of all Yayık butter samples were statistically different between day 1 and day 30 ($P<0.01$) (Table 5). These values were found to be consistent with earlier studies conducted by numerous researchers (Atamer et al. 2006; Şenel 2006; Şenel et al. 2011b).

Table 5 – Change in titratable acidity values of all Yayık butter samples on storage days*

	Storage days		
	1	***30***	***60***
Titratable acidity (°SH) [a]	$3.71 \pm 0.26^{b**}$	$4.13 \pm 0.26^{a**}$	$3.87 \pm 0.12^{ab**}$

*: Values are the $\bar{X} \pm S_{\bar{X}}$ of all Yayık butter samples on storage days of 1st, 30th and 60th (n=12), ** Denotes the significance level of difference $P<0.01$

According to the results given in Table 5, the highest titratable acidity values were observed on day 30 and values decreased on day 60 ($P<0.01$). A significant portion of lactose is converted to lactic acid by yogurt bacteria during yogurt production (Özer 2010) and after churning, some of the microorganisms involved in the fermentation and a small amount of residual lactose pass into the Yayık butter, resulting in a limited formation of lactic acid. Therefore, the small change in the values and a decrease on day 60 could be due to the existence of limited residual lactose in Yayık butter samples. Additionally, the increase in titratable acidity might be associated with the inhibition activity of mold/yeast on lactic acid bacteria (Kılıç 2010). In this study, although no sensorial defect related to mold/yeast was perceived on the 30th day of storage an increase in titratable acidity was detected in all samples (Table 3).

The mean values for the acid degree for Yayık butter samples during the storage period ranged from 1.70-1.75 mg KOH/g fat (Table 4). The acid degree value indicates the extent of the lipolysis which is the reaction of hydrolyzation of triglycerides to free fatty acids (FFAs) by lipase. The lipolysis of butter is one of the determinants of the shelf life of butter since some aroma/flavor defects (rancid flavor) can occur related to the extent of this reaction (Atamer et al. 2006). The storage and processing conditions and the quality of the cream are among the factors contributing to this reaction (Fardet et al. 2019). It was concluded that the rancid taste and aroma can occur when the acid degree value in cream butter exceeds 1.36 mg KOH/g fat and 1.53 mg KOH/g fat by Atamer (1983) and Ergin (1978), respectively. The panelists detected aroma/flavor defects when the acid degree values reached 1.08 mg KOH/g fat (Şenel et al. 2010). The threshold values mentioned here are about two times higher than those stated by Downey (1975). According to Table 4, acid degree values were higher than those reported by Sagdic et al. (2002) and Sagdic et al. (2004) but only a mild rancid flavor was perceived by the panelists in C samples on the 60th day of storage. The presence of $C_{(4:0)}$ and $C_{(6:0)}$ mainly among the short-chain fatty acids was associated with butter rancidity (Walstra et al. 1999). The possible reason for the perceived milk rancid flavor in sample C could be explained as the highest amount of $C_{(4:0)}$ and $C_{(6:0)}$ fatty acids during the storage period was detected in sample C (41.88 ppm) and followed by samples B (40.64 ppm), A (40.52 ppm) and D (39.96 ppm), respectively (Table 7).

According to Table 4, all Yayık butter samples were of good quality in terms of peroxide values ranging from 0.24-0.27 meq O_2/kg fat. The peroxide value is the indicator of oxidative degradation of butter, limiting the shelf-life of high-fat dairy products due to sensorial defects. The oxidation reaction primarily occurs in the presence of unsaturated fatty acids and oxygen (Şenel & Atamer 2015). The carbonyl compounds formed due to oxidation cause oxidized flavor in the products and are especially detected when the limits of peroxide value reach 2.0 meq O_2/kg fat (Downey 1975). The sensory threshold level for peroxide value in butter was reported as 6.3 meq O2/kg fat (Pearson 1974). However, Atamer & Sezgin (1984) and Atamer et al. (2006) expressed no correlation between the peroxide value and the aroma/flavor of butter. Peroxides do not effect the flavor directly since they decompose to carbonyl compounds that are responsible for the oxidized flavor defects in the products. Higher peroxide values occur in high-fat products indicating weaker oxidative stability. However, factors such as the oxygen concentration, the existence of metals (Cu^{+2}, Fe^{+3}), the presence of antioxidant substances and the water activity etc. affect this reaction and makes the interpretation of the results more challenging (Şenel & Atamer 2015).

3.3. Free fatty acids (FFAs) composition

3.3.1. Free fatty acids (FFAs) composition of yogurt

The FFA composition of yogurt samples is given in Table 6. The highest amount of short-chain saturated FFAs was observed in YB yogurt followed by YC, YD, and YA, in order. The concentration of medium and long-chain FFAs in all yogurt samples were close to each other and the highest amount was observed in YC (511.50 ppm) followed by YB (488.30 ppm), YD (414.40 ppm), and YA (411.28 ppm). The YC and YA samples were rich in unsaturated FFAs with values of 210.54 ppm and 210.13 ppm, respectively (Table 6). The FFA formation occurs during fermentation by lactic acid bacteria and the extent of this reaction depends on the strain (Mantzourani et al. 2022). The proto-cooperation reaction occurs between two yogurt bacteria *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* was found responsible for milk acidification, aromatic compounds, FFA formation, and organoleptic characteristics of yogurt and Yayık butter (Guler & Park, 2010). Based on the total amount of FFAs, it can be concluded that among the isolated strains, the combination of 27St/ALb used in YB yogurt

and among the commercial cultures CH1 used in YC yogurt favored FFAs formation mostly. The FFA content of yogurt samples is closely linked with its organoleptic characteristics and health effects in humans depending on their saturation degree, their chain length (short, medium, and long), and storage period (Gu et al. 2021; Mantzourani et al. 2022). Mantzourani et al. (2022) reported that higher amounts of FFA result in flavor defects in the products mainly associated with the increase in short-chain FFAs (Ozcan et al. 2016). Among short-chain FFAs, the butyric acid, known as the principal flavor component of butter, was observed in the highest amount in YB and followed by YC, YD, and YA, in order. Dairy products rich in short and medium-chain FFAs are associated with many health benefits. Short-chain FFAs are easily digested due to their lower melting point than the human body and are used as an energy source. Additionally, they are known with their antiviral and antimicrobial activities, especially butyric acid which has cancer-inhibiting activity (Ozcan et al. 2016).

Table 6 - The FFA composition of yogurt samples on the 1st day of storage (n=3)

	Yogurt samples ($\bar{X} \pm S_{\bar{X}}$)			
FFA (ppm)	***YA***	***YB***	***YC***	***YD***
Butyric ($C_{4:0}$)	6.01 ± 0.49	13.53 ± 5.10	11.7 ± 2.57	10.63±2.98
Caproic ($C_{6:0}$)	6.44 ± 0.44	12.36 ± 3.60	11.64 ±3.94	12.27±4.45
Caprylic ($C_{8:0}$)	4.68^{b*} ± 0.12	9.51^{a*} ±1.82	7.61^{a*}±2.40	7.25^{a*}±2.40
Capric ($C_{10:0}$)	8.55^{b*}±2.82	17.72^{a*}±6.59	20.44^{a*}±7.92	16.25^{a*}±5.91
∑($C_{4:0}$- $C_{10:0}$)	**25.86**	**53.12**	**51.39**	**46.40**
Lauric ($C_{12:0}$)	12.43^{b**} ±5.25	28.0^{a**}±12.30	34.50^{a**}±14.50	25.80^{ab**}±11.20
Myristic ($C_{14:0}$)	41.38±9.74	64.00±34.90	77.5±42.70	59.70±33.30
Palmitic ($C_{16:0}$)	211.97±1.80	277.80±97.50	274.00±146.00	221.00±121.00
Stearic ($C_{18:0}$)	145.50±26.40	118.50±51.90	125.50±62.70	107.90±57.70
∑($C_{12:0}$- $C_{18:0}$)	**411.28**	**488.30**	**511.50**	**414.40**
Oleic ($C_{18:1}$)	191.70±37.00	145.20±68.90	166.70±86.60	132.20±72.40
Linoleic ($C_{18:2}$)	18.43^{c**}±2.04	35.73^{b**}±1.41	43.84^{a**}±1.05	39.21^{ab**}±0.54
∑($C_{18:1}$- $C_{18:2}$)	**210.13**	**180.93**	**210.54**	**171.41**
Total FFA	**647.04**	**722.35**	**773.43**	**632.21**

Values are the $\bar{X} \pm S_{\bar{X}}$ of all Yogurt samples used in Yayık butter production on the 1st day of storage prior to Yayık butter production (n=3), Yogurt samples: * P <0.05 /** P<0.01 / $^{a-c}$: Different letters in the same row indicate significant differences among groups. YA: Yogurt inoculated by isolated strains of 27St/27Lb culture combination; YB: Yogurt inoculated by isolated strains of 27St/ALb culture combination; YC: Yogurt inoculated by commercial culture of CH1; YD: Yogurt inoculated by commercial culture of Y080.

The linoleic acid ($C_{18:2}$) content of YA differed significantly from YB, YC, and YD within the unsaturated fatty acids (P<0.05). Among saturated fatty acids, palmitic acid was found to be the most abundant in all samples with no significant difference (P>0.05) (Table 6). The lauric acid ($C_{12:0}$) content of YA yogurt was significantly different than YB and YC (P<0.05). The level of caprylic acid ($C_{8:0}$) was found to be the lowest among all fatty acids present in the yogurt samples and was significantly different in YA yogurt than in other yogurt samples (P<0.05). The most predominant FFAs in all yogurt samples were observed in the medium-long chain (palmitic acid ($C_{16:0}$), stearic acid ($C_{18:0}$), and in unsaturated FFAs as oleic acid ($C_{18:1}$) (Table 6). Similarly, as found by Şenel et al. (2011a) palmitic acid ($C_{16:0}$), oleic acid ($C_{18:1}$) and capric acid ($C_{10:0}$) were predominant in goat's milk yogurt samples.

3.3.2. Free fatty acids (FFAs) composition of Yayık butter samples

The distribution of free fatty acids (FFAs) in Yayık butter samples is shown in Figure 3, and the changes in free fatty acids (FFAs) content that occurred in the storage period of Yayık butter samples are given in Table 7. The highest free fatty acid accumulation was observed in Yayık butter samples A and B with isolated strains of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* and followed by C and D samples (Table 1). From the results, it is concluded that the selected strains used in A and B were thought to have higher lipolytic activity compared to the commercial starter cultures of the same bacteria (Figure 3).

Free fatty acid fraction (FFA) is generated through a lipolysis reaction catalyzed by the lipase enzyme (Collins et al. 2003). From Figure 3, the high-molecular-weight saturated fatty acids ($C_{12:0}$-$C_{18:0}$) were found to be predominant acids in all samples. These groups accounted for about 73% of the total free fatty acids. Similar results were reported in another study on Yayık butter (Şenel 2006). The extent of lipolysis, which results in higher levels of FFAs, limits the shelf life of dairy products due to undesirable changes in aroma/flavor characteristics during storage (Deeth et al. 1983). In dairy products, the rancid flavor is formed at high levels of FFAs (exceeding 1.5 mmol/L) (Deeth & Fitz-Gerald 2006).

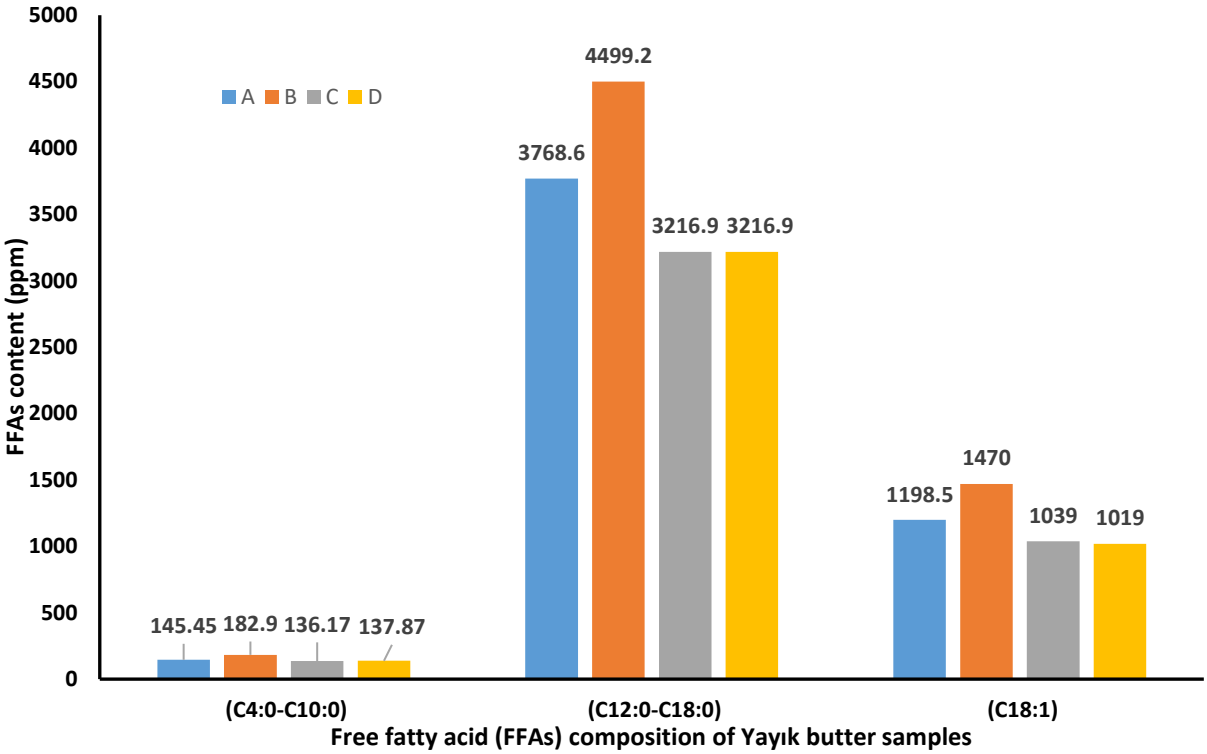

Figure 3- Means of the total amount of free fatty acids (FFAs) of Yayık butter samples during the storage period depending on the molecular weight (n=9)

Free fatty acids (FFAs): ($C_{4:0}$-$C_{10:0}$): Low-molecular-weight saturated fatty acids; ($C_{12:0}$-$C_{18:0}$): High-molecular weight saturated fatty acids; ($C_{18:1}$-$C_{18:2}$): Unsaturated fatty acids: Yayık butter samples; A: Yayık butter produced by yogurt (YA) inoculated by isolated strains of 27St/27Lb culture combination; B: Yayık butter produced by yogurt (YB) inoculated by isolated strains of 27St/ALb culture combination; C: Yayık butter produced by yogurt (YC) inoculated by commercial culture of CH1; D: Yayık butter produced by yogurt (YD) inoculated by commercial culture of Y080

In Yayık butter samples, nine different free fatty acids (FFAs) were extracted and identified (Table 7). Butyric acid (C_4), is a low-molecular-weight saturated, water-soluble bioactive element found in milk fat. It is responsible for the characteristic aroma and flavor of butter (Tamime & Robinson 1999), also known to reduce the risk of infectious intestine disease and lower the level of blood cholesterol (Deeth & Fitz-Gerald 2006; Van Immerseel et al. 2010). However, the level of butyric acid was found to be significantly lower in butter samples than in yogurt samples (Table 6) possibly due to the removal of a large proportion of water-soluble butyric acid during churning and washing the granules. According to the studies, the total amount of butyric acid in butter can vary between 3.12 – 8.83 ppm. Similar results have been obtained by Sağdıç et al. (2004) and Öztekin (2010). However, in a study conducted by Şenel (2006), the concentration of butyric acid was found to be higher than in our study, varying from 23.80 ppm on the first day of storage to 15.85 ppm at the end of the storage.

During the storage period, FFAs showed irregular changes in Yayık butter samples except for the amount of caproic acid ($C_{6:0}$) in sample C, in which a very slight decrease was observed. Similar to Sağdıç et al. (2004), our study also found that the most predominant FFAs in Yayık butter samples were palmitic acid ($C_{16:0}$), oleic acid ($C_{18:1}$), stearic acid ($C_{18:0}$) and myristic acid (C14:0). Furthermore, neither of the two unsaturated fatty acids linoleic acid ($C_{18:2}$) and linolenic acid ($C_{18:3}$) were detected in Yayık butter samples.

Table 7 - Changes in free fatty acid (FFAs) composition of Yayık butter samples during storage period*

FFAs (ppm)	*Storage Day*	*Yayık butter samples ($\bar{X} \pm S_{\bar{X}}$)*			
		A	***B***	***C***	***D***
Butyric ($C_{4:0}$)	1	3.12 ± 0.05	3.35 ± 1.14	4.44 ± 0.47	4.74 ± 0.21
	30	4.25 ± 1.74	6.83 ± 2.33	4.32 ± 0.92	3.38 ± 0.34
	60	5.47 ± 0.10	6.22 ± 0.10	5.23 ± 0.90	4.71 ± 0.10
Caproic (C6:0)	1	8.13 ± 0.91	10.62 ± 3.69	9.76 ± 3.59	9.42 ± 3.55
	30	8.20 ± 1.47	12.70 ± 2.83	9.51 ± 0.74	9.96 ± 0.65
	60	11.35 ± 1.56	9.92 ± 0.54	8.62 ± 2.54	7.75 ± 3.32
Caprylic ($C_{8:0}$)	1	21.17 ± 2.22	31.90 ± 13.20	23.80 ± 10.6	25.40 ± 10.90
	30	20.43 ± 0.05	36.80 ± 10.5	27.15 ± 2.20	29.05 ± 2.27
	60	29.77 ± 4.97	26.27 ± 2.61	21.61 ± 8.63	21.49 ± 9.22
Capric ($C_{10:0}$)	1	105.3 ± 10.09	134.2 ± 49.6	91.50 ± 45.00	95.80 ± 47.30
	30	91.39 ± 2.62	156.8 ± 37.00	111.8 ± 6.61	117.44 ± 4.02
	60	127.8 ± 13.90	113.14 ± 5.69	90.70 ± 32.30	84.00 ± 35.20
Lauric ($C_{12:0}$)	1	209.80 ± 19.80	264.90 ± 90.70	168.10 ± 82.80	176.40 ± 87.70
	30	190.10 ± 10.70	304.00 ± 69.60	218.70 ± 14.10	228.43 ± 7.16
	60	256.40 ± 14.20	224.16 ± 2.98	173.00 ± 60.30	161.30 ± 66.50
Myristic ($C_{14:0}$)	1	597.80 ± 70.30	774.00 ± 258.00	484.00 ± 239.00	503.00 ± 251.00
	30	550.70 ± 46.50	891.00 ± 193.00	633.90 ± 45.20	651.70 ± 27.60
	60	754.00 ± 30.00	653.41 ± 9.39	503.00 ± 178.00	463.00 ± 193.00
Palmitic ($C_{16:0}$)	1	2060 ± 209	2638 ± 895	1631 ± 804	1713.00 ± 851
	30	1943 ± 225	3060 ± 659	2147 ± 167	2229.40 ± 85.90
	60	2510 ± 113	1869 ± 324	1708 ± 585	1563.00 ± 644
Stearic ($C_{18:0}$)	1	707.20 ± 77.90	933.00 ± 349.00	594.00 ± 289.00	617.00 ± 304.00
	30	675.10 ± 72.20	1134.00 ± 246.00	759.80 ± 43.70	795.60 ± 22.50
	60	851.40 ± 41.70	752.90 ± 21.40	631.00 ± 219.00	549.00 ± 229.00
Oleic ($C_{18:1}$)	1	1161. 00 ± 106.00	1501.00 ± 563.00	919.00 ± 444.00	939.00 ± 464.00
	30	1065.00 ± 105.00	1711.00 ± 430.00	1206.20 ± 80.60	1259.70 ± 10.30
	60	1369.50 ± 65.30	1199.00 ± 36.20	993.00 ± 337.00	859.00 ± 352.00

*: Values are the $\bar{X} \pm S_{\bar{X}}$ of all Yayık butter samples on storage days of 1st, 30th, and 60th (n=9), Yayık butter samples; A: Yayık butter produced by yogurt (YA) inoculated by isolated strains of 27St/27Lb culture combination; B: Yayık butter produced by yogurt (YB) inoculated by isolated strains of 27St/ALb culture combination; C: Yayık butter produced by yogurt (YC) inoculated by commercial culture of CH1; D: Yayık butter produced by yogurt (YD) inoculated by commercial culture of Y080

3.4. The volatile compound distribution of the samples

3.4.1. Volatile compounds of yogurt samples

The volatile compounds of yogurt samples (YA, YB, YC, YD) are presented in Table 8. In total, twelve volatile compounds were identified, including four acids, six ketones, one aldehyde, and one ester (Table 8). Acetaldehyde is one of the most important carbonyl compounds produced by fermentation, contributing to the typical yogurt aroma (Rascón-Díaz et al. 2012) and giving fresh and green flavor (Tian et al. 2020). The concentration of acetaldehyde in yogurt ranges from 2 to 41 mg/L, influenced by the difference in the LAB strains and processes during fermentation (Li et al. 2024). However, a study reported that the yogurt develops its ideal flavor only if the concentration of acetaldehyde is greater than 8 mg/L (Chen et al. 2017). In yogurts inoculated with isolated-strains A and B yogurt samples, the acetaldehyde was not detected and only C and D yogurts got acetaldehyde in amounts of 8.31 mg/kg and 8.80 mg/kg, respectively. This concentration is adequate for a good flavor. In accordance with our findings, a study conducted by Şenel et al. (2011a) reported that the level of acetaldehyde in fresh yogurt was 9.11 mg/kg. This level remained stable for up to 45 days of storage. The starter culture effect on acetaldehyde formation is concluded by Gundogdu et al. (2020). Li et al. (2024) associated the production of the key flavor component in yogurt with the use of *Streptococcus. thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* as dominant starter cultures. However, yogurt fermented solely with *Lactobacillus casei* lacks the characteristic flavor compounds of yogurt.

As a result, our findings can be related to the higher metabolic activity of the commercial yogurt starter bacteria. Some researchers reported the loss of acetaldehyde in yogurt during the storage period. It was reported that acetaldehyde is converted to acetic acid and ethanol depending on pH and water activity (Gundogdu et al. 2020).

Table 8- The volatile compounds of yogurt samples on the 1st day of storage (ppm) (n=3)

	Yogurt samples			
Volatile compounds (mg/kg)	***YA***	***YB***	***YC***	***YD***
2,3-Butanedione	6.00	103.5	31.24	nd
2,3-Pentanedione	429.18	nd	nd	nd
2-Butanone	4.30	nd	nd	nd
2-Butanone, 3-hydroxy	17.73	26.21	28.88	17.94
2-Nananone	nd	nd	3.73	nd
2-Pentanone, 3-hydroxy	81.93	nd	nd	nd
2-Undecanone	nd	nd	nd	10.46
Acetaldehyde	nd	nd	8.31	8.80
Acetic acid	120.70	5.10	17.47	30.60
Butyric acid	54.36	68.61	54.03	48.74
Hexanoic acid	66.07	69.45	48.82	47.94
Octanoic acid	220.02	nd	nd	nd

Values are the means of all Yogurt samples used in Yayık butter production on the 1st day of storage prior to Yayık butter production (n=3), Yogurt samples: YA: Yogurt inoculated by isolated strains of 27St/27Lb culture combination; YB: Yogurt inoculated by isolated strains of 27St/ALb culture combination; YC: Yogurt inoculated by commercial culture of CH1; YD: Yogurt inoculated by commercial culture of Y080. nd: non-detected.

The acetic acid content of YA was quite higher than the other yogurt samples possibly due to this conversion reaction of acetaldehyde to acetic acid. The following compounds - 2-Butanone 3-hydroxy (acetoin), acetic acid, butyric acid, and hexanoic acid - were identified as common volatile compounds in all yogurt samples. A study reported that the starter culture had no effect on 2-butanone content, but storage time was found to have a significant impact (Gundogdu et al. 2020). 2,3-Butanedione (diacetyl) was identified in all yogurt samples except for YD. This common carbonyl compound is typically found in yogurt and butter, and its concentration depends on the specific combination of cultures used. In other words, it depends on the strains involved. Diacetyl is a volatile compound naturally produced by LAB and is responsible for the typical butter aroma in fermented dairy products, such as cheeses, butter, and yogurts (Papagianni 2012). Many microorganisms are able to produce diacetyl, such as Streptococcus, Leuconostoc, former-Lactobacillus, and other microbial genera, in quantities ranging from 0,4 to 8.2 g/L (Chen et al. 2017). In our study, the starter culture combination used in YB had a higher capacity to produce 2,3-Butanedione (diacetyl).

3.4.2. Volatile compounds of Yayık butter samples

The distribution of a total of thirty-one volatile compound detected in Yayık butter samples was summarized in Table 9. These compounds were classified into 9-acids (the most common includes acetic acid, benzene acetic acid, butyric acid, hexanoic acid-2-methyl, pentanoic acid-2-methyl, 4-hydroxymandelic acid, and peracetic acid), 4 alcohols (heptanol, hexanol, hexane, octadecane, methane, heptane, dodecane), 8-ketones (2-heptanone, methyl-ethyl-ketone, 2-butanone, 2-butanone hydroxyl, 2-nonanone, 2-octanone, 2-pentanone, 2-butanedione), 3 aldehydes (acetaldehyde, isobutyraldehyde, and hexanal), as well as seven unknown compounds. However, only seven volatile compounds were found to be common in all the Yayık butter samples (Figure 4).

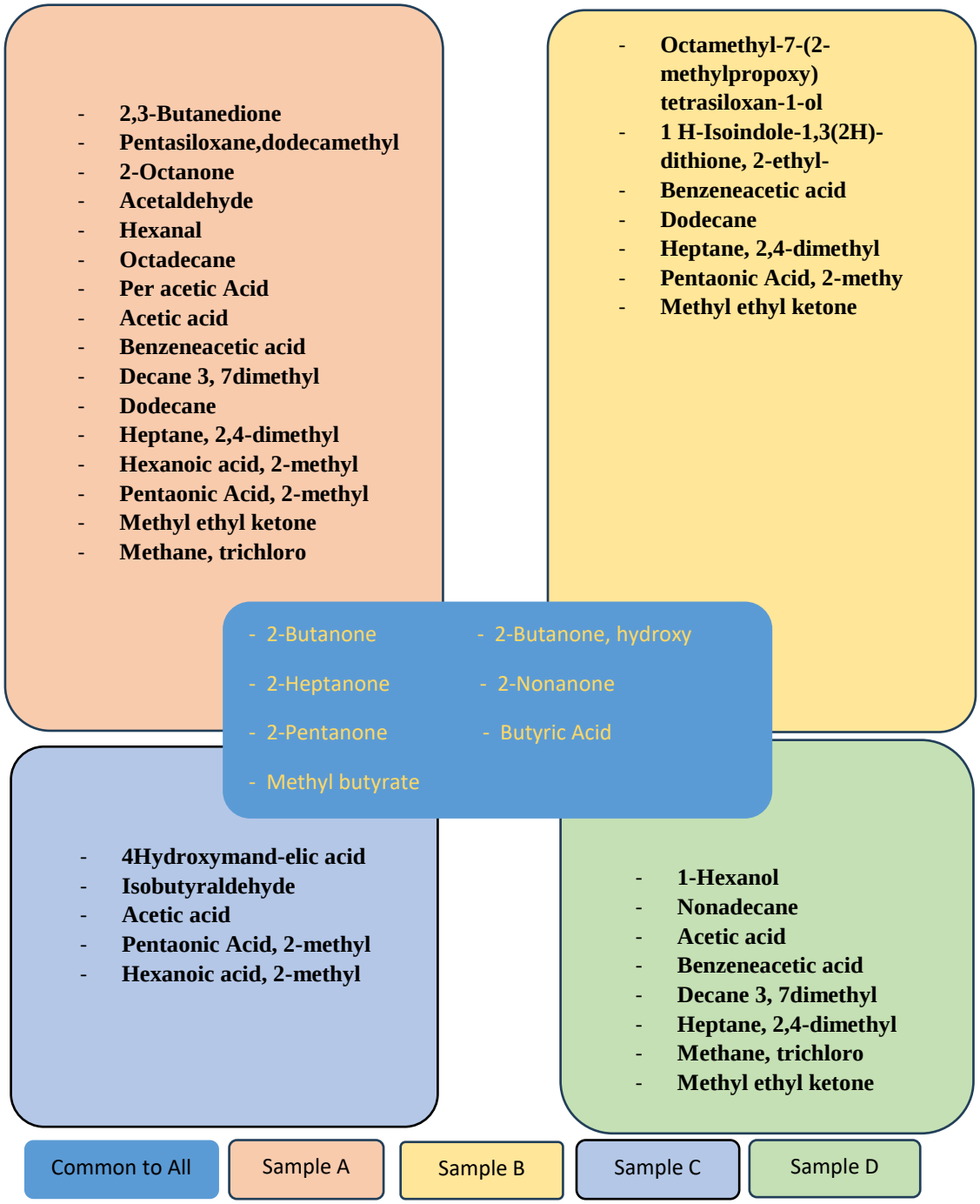

Figure 4 - Distribution of volatile compounds in Yayık butter samples

The aroma of the butter can vary based on different factors such as the breed of animal, their diet, the season, the raw material used (such as milk, yogurt, and cream), the production method, and the storage conditions. The characteristic aroma of butter is produced during storage, as a result of various biochemical reactions that occur due to microbial, enzymatic, and chemical transformation of milk components such as lipolysis, proteolysis, glycolysis, and pyruvate metabolism (Vanbergue et al. 2017).

Table 9- The volatile compounds of Yayık butter samples (n=3)

Volatile compounds (mg/kg)	*Storage Day*	*Yayık butter samples*			
		A	***B***	***C***	***D***
Hexanol	1	nd	nd	nd	nd
	30	nd	nd	nd	21.08
	60	nd	nd	nd	nd
1H-Isoindole-1,3(2H) dithione,2 ethyl-	1	nd	nd	nd	nd
	30	nd	20.65	nd	nd
	60	nd	nd	nd	nd
2,3-Butanedione	1	nd	nd	nd	nd
	30	14.18	nd	nd	21.08
	60	nd	nd	nd	nd
2-Butanone	1	43.36	78.15	81.25	118.34
	30	31.71	107.95	349.41	206.44
	60	76.73	nd	197.52	56.30
2-Butanone, hydroxy	1	7.68	4.12	27.76	25.64
	30	15.98	78.36	62.74	58.92
	60	40.63	nd	40.26	nd
2-Heptanone	1	64.38	27.36	39.05	32.25
	30	79.37	65.75	131.99	96.27
	60	nd	42.19	121.21	74.56
2-nonanone	1	nd	nd	nd	nd
	30	nd	nd	nd	42.33
	60	368.55	35.25	56.39	nd
2-Octanone	1	nd	nd	nd	nd
	30	nd	nd	nd	nd
	60	26.56	nd	nd	nd
2-Pentanone	1	nd	nd	nd	nd
	30	nd	nd	nd	43.57
	60	483.39	125.19	60.35	32.05
4-Hydroxymandelic acid, ethyl ester,di-TMS	1	nd	nd	nd	nd
	30	nd	nd	85.58	nd
	60	nd	nd	nd	nd
Acetaldehyde	1	3.11	nd	nd	nd
	30	nd	nd	nd	nd
	60	nd	nd	nd	nd
Acetic acid	1	49.58	nd	nd	nd
	30	49.57	nd	nd	75.49
	60	nd	nd	21.91	nd
Benzeneacetic acid	1	nd	nd	nd	nd
	30	nd	nd	nd	95.18
	60	27.20	458.87	nd	nd
Butyric acid	1	66.76	10.85	nd	47.44
	30	66.76	nd	33.50	34.71
	60	53.87	57.75	27.0	40.81
Carbon disulfide	1	nd	nd	43.23	nd
	30	nd	nd	nd	nd
	60	nd	nd	16.70	nd
Decane,3,7-dimethyl-	1	43.55	nd	nd	nd
	30	nd	nd	nd	19.49
	60	102.32	nd	nd	nd
Dodecane	1	102.38	nd	nd	nd
	30	nd	12.71	nd	nd
	60	38.18	nd	nd	nd
Heptane,2,4dimethyl-	1	74.9	nd	nd	nd
	30	nd	104.53	nd	23.20
	60	109.16	16.85	nd	19.17
Hexanal	1	nd	nd	nd	nd
	30	nd	nd	nd	nd
	60	28.11	nd	nd	nd
Hexane	1	nd	nd	nd	nd
	30	142.90	177.47	nd	nd
	60	70.03	nd	nd	68.10
Hexanoic acid	1	62.32	nd	29.78	nd
	30	57.49	nd	37.57	36.31
	60	nd	10.46	27.07	26.88

Table 9(Continue)- The volatile compounds of Yayık butter samples (n=3)

Volatile compounds (mg/kg)	*Storage Day*	*Yayık butter samples*			
		A	***B***	***C***	***D***
Hexanoic acid, 2 methyl-	1	16.55	nd	nd	nd
	30	nd	nd	nd	nd
	60	nd	nd	32.47	nd
Isobutyraldehyde	1	nd	nd	nd	nd
	30	nd	nd	nd	nd
	60	nd	nd	30.97	nd
Methane, trichloro-	1	nd	nd	nd	32.47
	30	11.73	nd	nd	nd
	60	nd	nd	nd	nd
Methyl butyrate	1	66.20	44.31	75.26	65.64
	30	46.84	nd	nd	100.45
	60	nd	nd	nd	nd
Methyl ethyl ketone	1	nd	19.47	nd	nd
	30	nd	nd	nd	80.43
	60	50.74	nd	nd	31.25
Nonadecane	1	nd	nd	nd	nd
	30	nd	nd	nd	65.27
	60	nd	nd	nd	nd
Octadecane	1	nd	nd	nd	nd
	30	nd	nd	nd	nd
	60	27.97	nd	nd	nd
Pentanoic acid, 2methyl-	1	27.92	21.45	35.36	nd
	30	nd	nd	nd	nd
	60	13.36	nd	6.80	nd
Pentasiloxane, dodecamethyl-	1	29.62	nd	nd	nd
	30	nd	nd	nd	nd
	60	nd	nd	nd	nd
Per acetic acid	1	27.95	nd	nd	nd
	30	nd	nd	nd	nd
	60	nd	nd	nd	nd

Yayık butter samples; A: Yayık butter produced by yogurt (YA) inoculated by isolated strains of 27St/27Lb culture combination; B: Yayık butter produced by yogurt (YB) inoculated by isolated strains of 27St/ALb culture combination; C: Yayık butter produced by yogurt (YC) inoculated by commercial culture of CH1; D: Yayık butter produced by yogurt (YD) inoculated by commercial culture of Y080. nd: non-detected

Among all the thirty-one volatile compound, ketones were the predominant chemical groups present in all samples during the storage period. Specifically, 2-butanone and 2-heptanone were the most abundant ketones detected in all butter samples nearly observed in all storage periods except for B on the 60th day for 2-Butanone and except for A on the 60th day for 2-Heptanone (Table 9). Ketones are also identified in fresh cream butter (Lee 2020) and cheeses (Qian & Reineccius 2002). Sample C was the richest Yayık butter in 2-Butanone and 2-Heptanone (349.41 mg/kg and 131.99 mg/kg on the 30th day of storage, respectively). Similar to our findings, Gundogdu et al. (2020) identified 11 ketones in butter samples, with the most abundant ones being 2-pentaone, 2-heptanone, acetoin, and 2-nonanone. The 2-heptanone is formed as the result of beta-oxidation of saturated fatty acids during thermal processing (Peterson & Reineccius 2003). Lactones are mainly responsible of the butter's creamy, fruity, or otherwise pleasant odors while sulfur-containing compounds contribute to its "corn-like" and "garlic" odor and dodecanoic acid gives it a "soapy" odor (Mallia 2008). Butyric acid and hexanoic acid were the most abundant carboxylic acids released from triglycerides and found in all Yayık butter samples. Hexanoic acid has been reported as a critical odorant in different cheese types, mainly cheddar cheese (Christensen & Reineccius 1995) and Grana Padano (Moio & ADDEO 1998). The highest concentration of butyric acid was detected in sample A and followed by sample D on all storage days (Table 9). Contrary to these results, Şenel et al. (2011b) found that only butyric acid was detected in trace amounts in all Yayık butter samples. Acetic acid produced by lactic starter cultures was detected in Yayık butter samples A, C, and D during storage (Figure 4, Table 9). The importance of acetic acid to the flavor of dairy products was established by Lozano et al. (2007) for butter, Delgado et al. (2010) for cheese, Bendall (2001) for milk, and Pan et al. (2014) for fermented milk. In Yayık butter samples pentanoic acid 2-methyl, benzene-acetic acid, hexanoic acid, 2-methyl, and peracetic acid were detected in lower amounts (Table 9). Hexanoic acid is associated with the pungent odor of blue cheese. In contrast, acetic acid, which is a result of lactose, lactate and citrate metabolism, has a pungent, vinegary acidic odor in dairy products (Dan et al. 2018).

In this study, only three aldehydes were detected in Yayık butter samples. Two of these aldehydes were found in sample A on the 1st day, and 60th days. Iso-butyraldehyde was only detected in sample C on the 60th day of storage (Table 9). Aldehydes

are frequently detected in dairy products and tend to convert into acids or alcohols such as methyl-butanal and hexanal during production (Afzal et al. 2012).

3.5. Sensory properties of Yayık butter samples

The sensory evaluation results of Yayık butter samples on storage days are presented in Figure 5. According to the results, the use of different starter culture combinations and storage periods as well as the interaction between different culture combinations and storage periods, did not have a statistically significant effect on all sensory attributes analyzed (P>0.05).

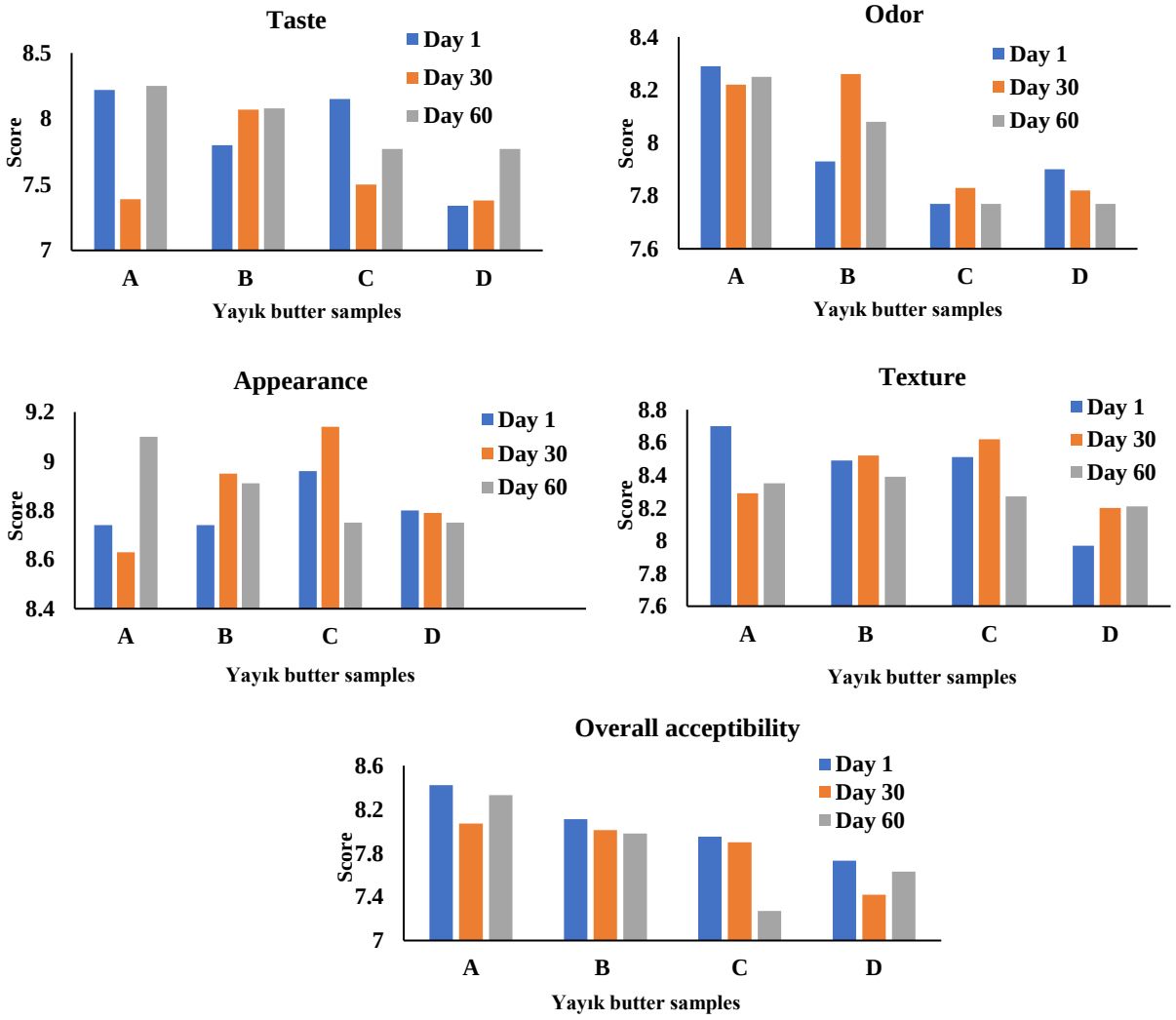

Figure 5 – Sensory properties of Yayık butter samples on storage days

Yayık butter samples; A: Yayık butter produced by yogurt (YA) inoculated by isolated strains of 27St/27Lb culture combination; B: Yayık butter produced by yogurt (YB) inoculated by isolated strains of 27St/ALb culture combination; C: Yayık butter produced by yogurt (YC) inoculated by commercial culture of CH1; D: Yayık butter produced by yogurt (YD) inoculated by commercial culture of Y080

The figure 5 reflects the mean values of each sensory attribute of Yayık butter samples during the storage period. In terms of taste, A got the highest score on the 1st and 60th days of storage followed by C on the 1st day and B on the 30th and 60th days. Sample D got the lowest scores all over the storage period. Yayık butter samples produced by isolated starter culture combinations showed superior odor properties as sample A got the highest score on the 1st and 60th days and sample B on the 30th day of storage. The samples produced by the commercial cultures (C and D) exhibited weaker odor characteristics. Regarding the appearance, Yayık butter samples (C and D) with commercial cultures, exhibited more uniform changes during storage, however, sample D got the lowest scores. Nearly all the Yayık butter samples were evaluated with high scores (> 8.00) in texture and samples A, B, and C were almost similar, but sample D got the lowest score in texture. Sample A was evaluated with the highest scores in all storage days followed by sample B in terms of overall acceptability attribute. Commercial culture used Yayık butter samples (C and D) were least accepted by the panelists during the storage period (Figure 5). In cream butter and Yayık butter samples, free fatty acid content, peroxide value, and acid degree value play a crucial role in sensory attributes (Şenel et al. 2016). According to the sensory evaluation, no distinct sensory defect was reported by the panelists except sample C in

which only a slight rancid flavor was pronounced by the panelists on day 60. From Table 4, the moisture content of sample C was the highest among the samples which might lead to amplification in enzymatic and microbial activity causing the breakdown of fats into free fatty acids and the release of short-chain fatty acids (Deeth & Fitz-Gerald 1976). Additionally, the yogurt (YC) used in the production of C butter had the highest total amount of FFAs content (773.43 ppm) (Table 6). As previously reported by Mantzourani et al. (2022), the high amount of FFA can cause flavor defects in dairy products. Based on these results, it can be concluded that culture combinations prepared by isolated strains gave satisfactory results sensorially when compared with commercial cultures.

4. Conclusions

This study was conducted to observe the effect of using two different isolated strain combinations of yogurt bacteria and two commercial yogurt starter cultures in Yayık butter production, to identify the potential effects on its quality attributes during a storage period of 60 days. The acidification potential of commercial cultures used was superior to the isolated strains combined. Both culture types used in Yayık butter samples exhibited similar FFA formation potential with a slight variation in FFA concentration. The highest free fatty acid accumulation was observed in endemic isolated combined cultures. One of the isolated combined cultures had the highest butyric acid content. Butyric acid and hexanoic acid were the most abundant carboxylic acids while ketones were the predominant volatile compound detected in Yayık butter samples. Culture combinations prepared by isolated strains gave satisfactory sensory results when compared with commercial cultures. The use of different starter culture combinations creates various FFA profiles influencing the nutritional properties, biological value, and aroma potential of the final product. This could contribute to both physico-chemical and organoleptic richness in industrial applications. The results of this study could serve as preliminary data for the possible use of traditional sources of starter cultures in scientific and industrial areas. It also emphasizes the importance of preserving traditional dairy products that reflect the cultural heritage.

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