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RESEARCH ARTICLE

Determination of bioactive properties of *Capparis spinosa* fruits and use in production of Tulum cheese

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ABSTRACT

The focus of the present study was to evaluate the bioactive properties of *Capparis spinosa* (caper) fruits and to investigate some quality parameters of Tulum cheese with addition of caper. Analyses for the phytochemical components of caper fruit used in the production of Tulum cheese showed that it has high levels of bioactive components in terms of antioxidant activity, total phenolics, and flavonoids. It was determined that *C. spinosa*, which is used in the production of Tulum cheese, had a good radical scavenging activity (DPPH radical assay). Four experimental groups were arranged in the present study and analyzed on the 1, 30, 45, 60, and 90th day of ripening. T_C was designed as the control group, T_C_1, T_C_2 and T_C_4 groups contained 1, 2, and 4% of caper. According to the obtained results as the content of caper increased, dry matter ratios, ash, pH values, tyrosine and free fatty acid values in cheese increased significantly. The microbiological evaluation revealed that the caper affected positively *Lactobacillus* spp. counts. The highest aroma and taste scores among caper cheeses were given to the control group (T_C) Generally, caper did not have a negative effect on the textural profile of the product. These results revealed that *C. spinosa* contributes to the improvement of the bioactive potential as well as increasing the quality parameters of cheese.

Keywords: Caper; Bioactive properties; Functional dairy; Microbiological properties; Tulum cheese

INTRODUCTION

The fundamental function of a diet is to provide an abundance of nutrients to meet metabolic demands, while also promoting feelings of satisfaction and well-being in the consumer. However, diets not only fail to fulfill nutritional requirements but also affect various physiological functions and can play either a harmful or helpful role in certain conditions. Accordingly, the consumption and development of novel food products containing biologically active compounds have arisen to mitigate the risks of non-transmittable chronic diseases (Kaur et al., 2022). Along with the expanding of consumers' knowledge on the relationship between health and chronic diseases, the functional food "sector" has grown rapidly. Factors such as longer life expectancy, rise in healthcare costs, social costs on non-transmittable diseases, and the omnipresent inclination for a better quality of life have made this concept thrive (Baker et al., 2022).

Dairy, as a nutrient-rich product, can be utilized as a carrier for functional ingredients. In conformity with that, dairy products are attributed to be the 'lead player' in the buildout of functional foods (Ali et al., 2022). Cheese represents one of the most consumed dairy products, an important source of nutritional components such as essential amino acids and fatty acids (El-Sayed &Youssef, 2019). Tulum is one of the traditional cheeses in Turkey. Tulum is referred to the goat's skin that has been serving as a packaging material. Tulum cheese produced from milk with different fat ratios is classified as semi-hard cheese (Yilmaz et al., 2005). Among many compounds that can be integrated into dairy products to enhance their nutritious properties are spices and herbs. Their medicinal use dates to ancient Egypt, India, and China. There are various research results revealing the beneficial effects of herbs and spices derived from them on human health with their different properties and suggesting their use (El-Sayed & Youssef, 2019). The first recorded scientific research on the impact of herbs and spices as preservative dates back in the 1880s and revealed the antimicrobial properties of cinnamon

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oil against *Bacillus anthracis* spores (Tajkarimi et al., 2010). At present, due to the presence of various bioactive compounds, beyond their health benefits herbs and spices are defined as good substitutes for the synthetic antimicrobial agents used in food manufacturing. Functional application of a variety of herbs and spices in diverse forms (i. e. powder, fresh, extract, essential oils, etc.) in some dairy products has been successfully reported (El-Sayed & Youssef, 2019).

Caper as a polyphenol-rich plant has gained a lot of attention in the recent decades. *Capparis spinosa* is representative of species in the Capparidaceae family which has medicinal, nutritional, and economical importance (Zhang & Ma, 2018). *C. spinosa* which can grow in arid, semi-arid climate, stony, prone, and unproductive areas, is a perennial thorny and bushy type of hardy plant with deep roots that can grow horizontally and vertically (Sun et al., 2022). *C. spinosa* is distributed in most Mediterranean countries and is actively produced in many countries of the region, including Turkey (Zhang & Ma, 2018).

Recent studies have revealed that quercetin, one of the common components of *C. spinosa*, has a protective and therapeutic effect against COVID-19 (Aucoin et al., 2020; Sharaf et al., 2000). This situation makes the study more valuable in terms of producing a functional product. Plant fruits' phenolic content varies with genotype, habitat, and maturity, and their scavenging capacity depends on the concentration and unique composition of phenolic compounds (Molina et al., 2023; Öztürk et al., 2023). Although the antioxidant activity, total phenolic and flavonoid content of the plant parts of *C. spinosa* have been revealed by numerous studies (Aliyazicioglu et al., 2013; Grimalt et al., 2019; Tlili et al., 2017; Yue-lan et al., 2010), there are not enough research results on the bioactive components and properties of its fruit. Reckoning the high nutritious profile and positive properties of caper on health, their integration in Tulum cheese, represents a strategy to produce a new functional food with enhanced physico-chemical and sensorial properties. Previously, hardly any studies have reported the use of caper in the manufacture of Tulum cheese or other dairy products. The results obtained in the current study can be regarded as a good reference for subsequent research. The objective of this study is to assess the bioactive properties of *C. spinosa* and to produce a high-quality Tulum cheese with the addition of processed *C. spinosa* fruits, especially in terms of functional properties.

MATERIALS AND METHODS

Preparation of caper

Caper fruits with 5% salt content and an average diameter of 7 mm used in production were commercially supplied from Berrak Capers (Zey-Tur-San Gıda San. ve Tic. A.S, İzmir, Turkey). The caper supplied in brine were preserved until the production of Tulum cheese. Prior to production, the caper was kept in water to reduce the salt content and dried at the room temperature. After drying, they were grinded by using an electric mill, and the overall ratio was added to the cheese in different concentrations as seen in Table 1.

Phytochemical screening of caper

Extraction procedure

The fruits of *C. spinosa* were powdered after drying in the shade at room temperature. Powdered sample (1 g) was extracted with 10 mL of methanol (Merck 106009, Damstad, Germany) HCl (Merck 100314, Damstad, Germany) mixture (80%/1%) keeping in water-bath (Memmert model WB 14, Germany) at 60 °C for 3 days. The obtained extract was filtered through a Whatman no.1 filter and then concentrated with a rotary evaporator (ISOLAB, LB.IS.605.01.001) under reduced pressure at 50°C and the volume was adjusted to 50 mL with methanol. Extracts were stored at -20 °C until analysis (Tlili et al., 2017).

Antioxidant activity (DPPH radical scavenging assay)

The antioxidant capacity of the produced cheese samples was determined with the 1,1-diphenyl-2, picrylhydrazyl (DPPH) method as described by Miliauskan et al. (2004). In brief, 3 mL of a stock solution (6×10^{-5} M) was mixed with 77 µL of the extract and incubated for 15 min in the dark at ambient temperature. Simultaneously, a sample containing the same amount of methanol as the DPPH solution was used as blank. The absorbances were measured at 517 nm with a spectrophotometer device (ATI-Unicam, model UV-4, UK). In the present assessment, triplicates of all tests were performed. The radical scavenging activity was assessed with the formula as follows:

$$\text{Scavenging activity (\%)} = [(\text{A control} - \text{A sample})/\text{A control}] \times 100$$

where A-control and A-sample are the absorbance values of the control and the sample extracts, respectively. The DPPH radical scavenging activity is reported as the IC50 value, which is defined to be the concentration of the sample required to scavenge 50% of free radicals present in the experimental solution (Adebiyi et al., 2017).

Table 1: Concentration of *C. spinosa* in Tulum cheese groups

Group	Caper concentration (%)
T_C	-
T_C_1	1
T_C_2	2
T_C_4	4

Total phenolic content (TPC)

TPC of the extract was evaluated by the Folin-Ciocalteu method as described by Singleton et al. (1999). Gallic acid (Sigma-Aldrich, 5995-86-8) was used as a standard for the assessment. Following sample preparation, the alteration in color generated after incubation at 23°C for 2 h, was measured at a wavelength of 765 nm by using a UV visible spectrophotometer (ATI-Unicam, model UV-4, UK). The calibration curve was prepared by using the gallic acid between 0-200 mg/mL. The obtained TPC values were expressed as mg gallic acid equivalent/100 g of dry sample (GAE/100). All determinations were carried out in triplicate. For the calibration curve the following formula was utilized:

$$y= 0.0018x + 0.003 \; R^2= 0.9987$$

where x is the absorbance and y is the concentration as gallic acid equivalents.

Total flavonoids content (TFC)

TFC was determined using the method described by Zhizhen et al. (1999). Initially, 1 mL of the diluted sample extract was transferred to 4 mL of distilled water, and following that 0.3 mL $NaNO_2$ (5% v/v) was further transferred. Following a 5 min incubation, 3 mL of 10% $AlCl_3$ was added and the solution was incubated for 6 min. Afterwards, 2 mL of a 1M NaOH solution was added to the solution and the final volume of the mixture was made up to 10 mL with distilled water. The mixture was thoroughly mixed and the absorbance was measured against a blank at 510 nm by using a UV visible spectrophotometer (ATI-Unicam, model UV-4, UK). The final TFC was expressed as quercetin equivalent per gram dry weight (mg QE/g DW). All tests in this evaluation were performed in triplicate. The following formula was used for the calibration curve:

$$y= 0.0064x + 0.0041 \; R^2= 0.9864$$

where x is the absorbance and y is the concentration as quercetin equivalents.

Manufacture of tulum cheese

Prior to cheese production, raw milk, and caper (dry, wet, and brined) were subjected to both physico-chemical and microbiological evaluation. Ewe's milk used in cheese production was obtained from the local market. Four different cheese groups were produced (Table 1). Initially, raw milk was placed in stainless-steel vats, pasteurized for at 72 °C for 2 min, then cooled to 31-32 °C. After the milk was cooled a commercial starter culture (0.5%) and $CaCl_2$ (0.02%) were added. *Lactococcus lactis* subps. *lactis*, *Lactococcus lactis* subps. *cremoris* and *Streptococcus salivarius* subsp. *thermophilus* (Choozit™ Ra 22 Lyo 125 Dcu and Choozit™ Ma 16 Lyo 50 Dcu, Danisco, Istanbul, Turkey) were used as starter cultures. Initially, freeze dried cultures were inoculated into skimmed milk under aseptic conditions and kept at 30 °C until the pH decreased to approximately to 4.5-4.6. The culture, which was then kept at 4 °C was used in cheese production. Rennet (1/50.000) was supplied from Maxiren® 600 BF (FML Gıda Ltd Şti., Kayseri, Turkey) and was added to milk and kept for 90 min until curd formation. After the curd was cut into cubes it was transferred to a cotton bag and then allowed to drain for 24 h (2 kg of weight per1 kg curd). Following this step, the curd was broken into pea size, salted (2%) and caper powder was added to each group in the concentrations (w/w) as shown in Table 1. Afterwards, cheeses were left to drain under pressure for 24 h (3 kg of weight per 1 kg curd). Then the curd was cut a second time and resalted (2%) and drained for 24 h (3 kg of weight per 1 kg curd). At the end of this procedure, the curd was cut one final time, left to rest for 2 h and, uniformly mixed and tightly filled into sterile polypropylene containers. All cheeses were ripened at 4 ± 1°C for 90 days. The samples were subjected to the analysis on the 1, 30, 45, 60 and 90th days of ripening. Production and analysing were carried out in three repetitions (triplicate).

Microbiological analyses

Raw milk, caper and cheese samples (10 g each) were transferred aseptically to stomacher bags with 90 mL of peptone water and homogenised in a stomacher (IUL Instruments Masticator, Spain) for 60 s at ambient temperature. Appropriate serial dilutions were prepared in 0.1% peptone water solution (TS-4018912, Biolife, Italy), and bacterial populations were counted using selective mediums. Inoculated plates in duplicate were incubated and at the end of incubation only plates containing 30-300 colonies were evaluated. Microorganism counts of samples were expressed as log colony forming units per gram (log cfu_{10}/g) (ISO, 4833 2003). Total aerobic mesophilic counts were enumerated on plate count agar (Oxoid CM325, Fisher Scientific, Hampton, USA) incubated at 30±1 ºC for 48 h (ISO, 4833 2003). Yeast and mold counts were enumerated on potato dextrose agar (Difco B 13, New Jersey, USA). Plates were incubated at 22 ± 1 ºC and evaluated after 5 days (ISO, 21527-2 2018). Coliform counts were enumerated on violet red bile agar (Merck KGaA, Darmstadt, Germany, VM730375616). Plates were incubated at 30±1 ºC and evaluated after 24 h (ISO, 4832 2006). *Escherichia coli* identification was done on tryptone bile X–glucuronide medium (Oxoid CM 0945, Thermo Fisher, Massachusetts, USA). Initially, plates were incubated at 30 ºC for 4 h and then they were transferred to 44 ºC and incubated for 18 h (ISO, 4832 2006). *Lactobacillus* spp. counts were enumerated on MRS medium (Merck 1.10660, Darmstadt, Germany). Plates were incubated in

anaerobic conditions (Anaerocult A, Merck, Darmstadt, Germany) at 37 °C for 72 h (De Man et al., 1960). *Lactococcus* spp. counts were enumerated on M17 medium (Merck 1.15108, Darmstadt, Germany). Plates were incubated at 30±1 °C for 48 h (Choi et al., 2000). At the end of incubation as the above mentioned distinct typical colonies were determined as *E. coli*, *Lactobacillus* spp. and *Lactococcus* spp., respectively.

Physico-chemical analyses

Titratable acidity, pH, fat content, ash, dry mater, total nitrogen and protein content and a_w value were evaluated in accordance with (AOAC, 2019). Salt content was determined according to Mohr titration method (ISO, 2018). Tyrosine content was determined spectrophotometrically as described by (Gutiérrez-Méndez et al., 2019). This method depends on the interaction between tyrosine and the Folin-Ciocalteau reagent forming a blue coloured complex. Free fatty acid concentrations in cheese were determined as described by De Jong & Badings (1990) with slight modification and the results were expressed in oleic acid (%). Ripening Index (RI) was expressed as the percentage of soluble nitrogen in total nitrogen (TN) content by using the formula: (NPN/TN and NCN/TN) x 100 (Balabanova & Vlaseva, 2017).

Sensory analysis

Sensory evaluation was performed in accordance with ISO standard (ISO, 2016). The panelist group consisted of 10 trained members (5 females + 5 males) from the Department of Food Hygiene and Technology. Group scoring was performed in a well-lit, odor-free room at an optimal temperature (20°C). For all the panel sessions, samples of approximately 10 g each were placed in plastic plates coded with a letter corresponding to each group. The ratings were made on a 5-point hedonic scale (5 = very pleasant; 1 = very unpleasant). Panelists evaluated the appearance, texture, taste and odour of the cheese samples.

Textural analyses

Texture profile assessment (Brookfield texture analyzer; Brookfield Engineering, CT3-4500, V1.8 Build 31, USA) was used in two compression cycles and done to assess the texture of the produced cheese. The size of each tested sample was a height of 50 mm and a diameter of 45 mm. Samples were downsized to 10% of their original height with a delay of 10 seconds between compressions. The pre-evaluation, evaluation, and post-evaluation speeds were 2 mm/s, 2 mm/s, and 10 mm/s, respectively. A cylindrical metallic probe (TA41) with a diameter of 6 mm and a height of 35 mm was used. Seven parameters (hardness, springiness, resilience, chewiness, external adhesiveness, internal cohesiveness and gumminess) were examined in texture profile analysis (Golmakani et al., 2019; Romeih et al., 2002).

Statistical analyses

Production and analysing were carried out in three repetitions (triplicate). The statistical analysis of the data was carried out via One-Way Analysis of Variance (ANOVA) with mean comparison analysis conducted using Tukey's test. Statistical Package for the Social Sciences was used for the analyses. The level of significance was set at $p < 0.05$. The results are expressed as Mean ± Standard Deviation (SD).

RESULTS

Phytochemical screening results

Phytochemicals, which are the main responsible for the antioxidant activity of plants with a wide range of bioactive molecules, are accepted as active agents that promote, maintain, and repair health in cells and tissues in human body. The aim of this study is to examine the bioactive properties of *C. spinosa* as well as its influence on various quality measures when integrated to Tulum cheese. In the current study, the antioxidant activity, the total phenolic and flavonoid content of fruits of *C. spinosa* are shown in Table 2. Antioxidant activity level of caper was determined to be 44.43%. IC50 value of DPPH radical scavenging assay was 4.82 mg/mL DW. Total phenolic and flavonoid contents of caper was 155.93 mg GAE/100 g DW and 16.64 mg QE/g DW, respectively. In the research on bioactive properties of *C. spinosa* and *Capparis ovata* different results were obtained depending on plant species, their parts and extracts used in the analysis. Ghafoor et al. (2020) stated higher total phenolic and flavonoid content and antioxidant activity values in various parts of *C. spinosa* compared to caper fruits. Bouriche et al. (2011) stated that *C. spinosa* buds exhibited a stronger scavenging activity against DPPH radical (53 μg/mL) than obtained with the standard antioxidant BHT. However, in the current study, the bioactive component values of *C. spinosa* fruits were revealed at a higher rate than the others (Aksay et al., 2021; Allaith et al., 2016). This might be attributed to variables such as solvent, geographic origin, ambient stress, analytical technique, and processing.

Microbiological results

TAMC, coliform, *Lactobacillus* spp, *Lactococcus* spp and yeast and mould counts of raw milk used in production were 7.55, 5.93, 6.25, 7.44 and 7.17 as log10 cfu/ml, respectively. While coliform, *Lactobacillus* spp, *Lactococcus* spp could not be detected in caper, TAMB and yeast and mould counts were found at the levels of 4.38 and 3.72 as log10 cfu/ml, respectively. Table 3 shows the evolution of different microbiological counts throughout

Table 2: Bioactive properties of *C. spinosa* fruits

Variety	Antioxidant activity		Total phenolic content (mg GAE/100 g DW)	Total flavonoid content (mg QE/g DW)
	% Inhibition	IC50 (mg/mL DW)		
C. spinosa	44.43±0.82	4.82±0.35	155.93±2.86	16.64±0.21

Table 3: Comparison of changes in bacterial counts of Tulum cheese during ripening period (log cfu g^{-1})

	Evaluation days				
	1	30	45	60	90
TAMC					
T_C	8.70±0.10^{Cc}	9.32±0.01^{Aa}	8.98±0.12^{Bb}	8.95±0.11^{Bb}	9.00±0.11^{Cb}
T_C_1	8.83±0.19^{Ca}	9.03±0.15^{Ba}	8.93±0.19^{Ba}	8.97±0.16^{Ba}	9.07±0.15^{Ca}
T_C_2	8.99±0.02^{Bbc}	9.13±0.02^{Bb}	8.98±0.02^{Bd}	9.03±0.06^{Bc}	9.18±0.01^{Ba}
T_C_4	9.35±0.06^{Ad}	9.42±0.02^{Ad}	9.24±0.05^{Ac}	9.44±0.03^{Ab}	9.61±0.07^{Aa}
Coliform counts					
T_C	0.00^{C}	0.00	0.00	0.00	0.00
T_C_1	1.77±0.22^{A}	0.00	0.00	0.00	0.00
T_C_2	1.31±0.19^{B}	0.00	0.00	0.00	0.00
T_C_4	1.45±0.20^{B}	0.00	0.00	0.00	0.00
Yeast and moulds					
T_C	6.31±0.01^{Cc}	6.65±0.06^{Ab}	6.70±0.08^{Ab}	6.78±0.10^{Aa}	6.83±0.07^{Ba}
T_C_1	6.36±0.05^{Cc}	6.60±0.02^{Aab}	6.68±0.09^{Aa}	6.73±0.13^{Aa}	6.78±0.10^{Ba}
T_C_2	6.44±0.01^{Bd}	6.69±0.02^{Ac}	6.70±0.03^{Ac}	6.84±0.04^{Ab}	6.92±0.01^{Ba}
T_C_4	6.60±0.04^{Acd}	6.74±0.17^{Abc}	6.80±0.15^{Abc}	7.07±0.38^{Ab}	7.45±0.03^{Aa}
Lactobacillus spp.					
T_C	7.91±0.15^{Ac}	9.33±0.13^{Aa}	8.30±0.04^{Bb}	8.36±0.05^{Bb}	8.07±0.08^{Bc}
T_C_1	7.70±0.04^{Bd}	8.65±0.03^{Ba}	7.92±0.01^{Bc}	8.05±0.03^{Bb}	7.91±0.06^{Bc}
T_C_2	7.90±0.14^{Ab}	9.08±0.29^{ABa}	8.94±0.57^{Aa}	8.96±0.42^{Aa}	8.74±0.42^{Aa}
T_C_4	7.59±0.01^{Be}	9.41±0.01^{Aa}	7.99±0.02^{Bc}	8.36±0.01^{Bb}	7.77±0.02^{Bd}
Lactococcus spp.					
T_C	8.89±0.01^{Cb}	9.70±0.05^{Ca}	8.81±0.01^{Cc}	8.79±0.01^{Dc}	8.69±0.02^{Cd}
T_C_1	8.94±0.01^{Bd}	9.83±0.03^{Aa}	8.96±0.02^{Ad}	9.03±0.01^{Cb}	8.89±0.02^{Bc}
T_C_2	8.98±0.01^{Ac}	9.86±0.02^{Aa}	8.95±0.01^{Ad}	9.07±0.01^{Bb}	8.90±0.01^{Be}
T_C_4	8.70±0.02^{De}	9.60±0.04^{Ba}	8.85±0.03^{Bd}	9.12±0.04^{Ab}	8.98±0.02^{Ac}

Values are expressed as means±standard deviation (SD)
A-D: Values with different superscripts in the same column are significantly different (p<0.05)
a-e: Values with different superscripts in the same line are significantly different (p<0.05).

cheese ripening. Microbiological evaluation revealed that with the increase in caper content, the total aerobic mesophilic counts (TAMC) increase too. Among these groups, the lowest TAMC were observed in group T_C_1 meanwhile the highest in group T_C_4. TAMC variations in counts throughout ripening were higher in group T_C_4 compared to the other caper containing groups. Control group (T_C) when compared with the other groups, shows a significant difference with T_C_2 and T_C_4 groups, but not the T_C_1 group. The high TAMC were related to the high microbial load of the sheep milk used in cheese production. With the reduction in the salt content and added caper in dry form, there was a general increase in TAMC during ripening. On the other hand, due to both water absorption capacity of the dried caper and their initial bacteriological load, TAMC were higher in caper containing groups.

The evaluation of the yeast and mould counts exhibited that the control group (T_C) had the lowest counts, while they increased as the caper content increased. There were significant differences between the values determined at the beginning and end of ripening in terms of yeast and mold count in all groups ($p < 0.05$). The difference between counts in group T_C_1 and T_C_2 was significant ($p < 0.05$) on the last day of evaluation. Also, on the 90th day, the difference in counts between group T_C_4 and T_C, T_C_1 and T_C_2 groups was found to be statistically significant ($p < 0.05$). The Turkish Standard Institute TS 3001 has not set any criteria related to yeast and moulds for Tulum Cheese (Anonymus, 2006). Similarly, in many countries, there is no yeast and mould standard for Tulum cheese. There are more contamination tools in the production of Tulum cheese than other types of cheese, one of the most important of which is drying step of the product, which is carried out in open conditions.

Evaluation of coliform counts throughout ripening period, exhibited their presence in samples containing caper. Coliform counts appeared to be present in those groups

(T_C_1, T_C_2 and T_C_4) only in the first day of the ripening. The results demonstrate that *Escherichia coli* was not present in any of the groups, thereby assuring the microbiological safety of cheese. When all groups are considered, the highest *Lactococcus* spp. counts were detected on the 30th day of ripening. Except for group T_C_4, the counts in the last day of ripening were found lower compared to the first day. Results of *Lactococcus* spp. counts in group T_C_4, were statistically significant ($p < 0.05$). By the end of the ripening those groups (T_C and T_C_4) even though with a little difference revealed the lowest and highest counts, respectively.

It was determined that the *Lactobacillus* spp. counts of all groups on the final day of evaluation were higher compared to the first day of storage. The highest increase in *Lactobacillus* spp. counts appeared between the 1-30th days, meanwhile the highest decrease appeared between the 30-45th day. It was observed that the *Lactobacillus* spp. counts increased proportionally and numerically among caper containing groups. Out of them group T_C_4 exhibited the highest increase. Differences in *Lactobacillus* spp. counts of group T_C were found to be statistically significant throughout ripening period ($p < 0.05$). On the 90th day, a significant difference was found only between group T_C_2 and groups T_C, T_C_1 and T_C_4. The obtained data reveal that the caper used in Tulum cheese affected the *Lactobacillus* spp. counts. Similarly, Franceska et al. (2016) found that during the fermentation of caper berries, *Lactobacillus pentosus* was the dominant strain among the microbial communities.

Physico-chemical results

The pH, titratable acidity, dry matter, fat, protein and nitrogen values of sheep milk used in production were determined as 6.70, 0.22%, 20.30%, 9.12%.,5.45% and 0.85%, respectively. The moisture, total protein, ash and fat contents of caper fruits were established 81.4%, 1.8%, 5.2% and 0.8%, respectively. Mean values for dry matter, salt, salt in dry matter, ash, titratable acidity, pH, fat, fat in dry matter, tyrosine, nitrogen, protein, protein in dry matter, free fatty acids and ripening index of cheese samples are presented in Table 4. Throughout ripening period, the lowest dry matter value was determined on the 90th day (group T_C) meanwhile the highest was recorded on the 60th day (group T_C_4). A significant relationship was found between the changes in the dry matter ratios during cheese ripening in group T_C ($p<0.01$). On the other groups (T_C_1, T_C_2 and T_C_4) dry matter rates increased until the first 60 days and appeared to decrease in the last day. This might happen because during the ripening process, peptide bonds in S1-casein are broken and new ionic groups are generated. These ionic groups are significant in the water absorption capacities of proteins during low-temperature storage, resulting in a reduction in the dry matter content of cheeses (Gursoy & Kinik, 2010; Sarantinopoulos et al., 2002).

Among all analyse days, the lowest salt ratio was detected on the 1st day (group T_C) and the highest salt ratio on the 60th day (group T_C_2). There was a continuous increase in salt ratio in the first 45 days in groups T_C, T_C_1 and T_C_2. It was found that as the concentration of caper increased, the salt ratios also increased in the first day of ripening. The changes in the salt ratios of all the groups during ripening period were found statistically significant ($p < 0.05$). Considering the correlation between salt and dry matter, except for the 90th day of storage, a significant correlation was found ($p < 0.05$). On the last day of storage compared to the 60th day, the salt content of T_C, T_C_1 and T_C_2 groups decreased while the salt content of T_C_4 group is increase. Likely, differences in dry matter content impacted the extent to which salt penetrated the cheese. The high ratios of salt contents measured in this study were also observed in similar studies. It is likely that various factors, such as the brining of the cheese, the temperature during the brining process, and the cheese composition, determined these findings (Yerlikaya & Karagozlu, 2014).

When comparing the differences between the groups in terms of ash content, a significant relationship was found between groups in the first day of ripening ($p < 0.05$). It was observed that the ash content increased significantly with the increase in the concentration of caper ($p < 0.05$). Among these groups, the highest increase in ash content was in groups T_C_2 and T_C_4. The differences observed between groups were described as statistically significant ($p < 0.05$). Total mineral matter level of caper added to cheese can also affect the total ash content.

In terms of acidity, the highest acidity value on the 1st day was seen in group T_C_2 (0.92%) while the lowest in group T_C (0.81%). On the 90th day, acidity percentages of group T_C, T_C_1 and T_C_2 decreased, while the T_C_4 showed an increase of 0.01 ($p > 0.05$). An overall view of the pH values revealed variable values. In the following days of ripening, the pH values of the caper containing groups were significantly higher than the control group ($p < 0.05$). The pH values of the samples with caper began to increase in the 30th day as the content of caper increased. Looking at the results, it was found that pH exhibited the lowest value on the 45th day.

As the caper content increased, the fat content decreased. The lowest fat content corresponded to group T_C_4 (25.21%) while the highest belonged to group T_C (27.13%). On the last day of storage, a significant relationship was found in the fat content of group T_C

Table 4: Comparison of changes in physico-chemical parameters of Tulum cheese during ripening period

	Evaluation days				
	1	**30**	**45**	**60**	**90**
Dry matter					
T_C	58.44±0.03^{Cc}	59.30±0.03^{Db}	59.80±0.02^{Da}	58.33±0.04^{Dd}	58.11±0.05^{De}
T_C_1	58.50±0.04^{Ce}	59.53±0.05^{Cd}	60.10±0.01^{Cb}	60.43±0.02^{Ca}	59.75±0.03^{Cc}
T_C_2	59.89±0.08^{Bd}	60.03±0.02^{Bc}	60.95±0.03^{Bb}	61.13±0.02^{Ba}	60.87±0.06^{Bb}
T_C_4	60.49±0.03^{Ae}	61.23±0.07^{Ac}	61.34±0.07^{Ab}	61.46±0.02^{Aa}	60.95±0.04^{Ad}
Salt content					
T_C	3.99±0.02^{De}	4.14±0.01^{Dc}	4.24±0.01^{Da}	4.16±0.01^{Cb}	4.07±0.02^{Dd}
T_C_1	4.12±0.01^{Ce}	4.28±0.02^{Bb}	4.32±0.02^{Ba}	4.23±0.01^{Bc}	4.16±0.02^{Cd}
T_C_2	4.17±0.03^{Bd}	4.31±0.01^{Ac}	4.38±0.03^{Aa}	4.41±0.04^{Aa}	4.29±0.03^{Abc}
T_C_4	4.23±0.01^{Ab}	4.26±0.01^{Ca}	4.28±0.02^{Ca}	4.15±0.01^{Cd}	4.21±0.01^{Bc}
Ash content					
T_C	5.53±0.02^{Db}	5.56±0.02^{Db}	5.61±0.01^{Da}	5.59±0.01^{Da}	5.58±0.01^{Da}
T_C_1	5.71±0.01^{Cb}	5.86±0.03^{Cc}	6.19±0.02^{Ba}	6.25±0.07^{Ba}	6.18±0.04^{Ba}
T_C_2	5.85±0.03^{Bc}	6.00±0.03^{Bb}	6.05±0.03^{Ca}	6.01±0.04^{Ca}	5.95±0.05^{Cb}
T_C_4	6.76±0.05^{Ad}	7.16±0.05^{Ab}	7.29±0.04^{Aa}	7.18±0.05^{Ab}	7.02±0.06^{Ac}
Titratable acidity					
T_C	0.81±0.01^{Dd}	0.97±0.01^{Ac}	1.06±0.02^{Ab}	1.15±0.05^{Aa}	1.13±0.03^{Aa}
T_C_1	0.87±0.01^{Bd}	0.93±0.01^{Cc}	0.96±0.01^{Cb}	1.06±0.02^{Ca}	1.03±0.02^{Ca}
T_C_2	0.92±0.01^{Ae}	0.96±0.02^{Bd}	1.03±0.02^{Bc}	1.08±0.01^{Ba}	1.05±0.01^{Bb}
T_C_4	0.84±0.01^{Cc}	0.92±0.01^{Db}	0.94±0.01^{Da}	0.91±0.01^{Db}	0.92±0.01^{Db}
pH					
T_C	5.13±0.01^{Da}	4.75±0.01^{Db}	4.66±0.01^{Dd}	4.70±0.01^{Dc}	4.72±0.01^{Dc}
T_C_1	5.16±0.02^{Ba}	4.87±0.01^{Cc}	4.82±0.02^{Cd}	4.86±0.02^{Cc}	4.93±0.01^{Cb}
T_C_2	5.20±0.01^{Aa}	4.92±0.01^{Bc}	4.87±0.01^{Be}	4.89±0.01^{Bd}	4.96±0.01^{Bb}
T_C_4	5.15±0.02^{Ca}	4.94±0.02^{Ad}	4.98±0.02^{Ac}	5.11±0.02^{Ab}	5.10±0.02^{Ab}
Fat content					
T_C	27.13±0.14^{Ab}	28.18±0.20^{Aa}	28.16±0.23^{Aa}	28.13±0.27^{Aa}	27.11±0.16^{Ab}
T_C_1	26.11±0.11^{Bb}	26.13±0.13^{Db}	27.18±0.09^{Ca}	27.23±0.08^{Ba}	26.13±0.08^{Bb}
T_C_2	26.11±0.09^{Bb}	26.19±0.08^{Bb}	27.19±0.12^{Ba}	26.14±0.08^{Cb}	26.11±0.09^{Cb}
T_C_4	25.21±0.11^{Cb}	26.18±0.15^{Ca}	25.14±0.20^{Db}	25.11±0.23^{Db}	24.08±0.15^{Dc}
Tyrosine content					
T_C	3.09±0.01^{Ce}	3.27±0.02^{Ad}	3.42±0.01^{Ac}	3.58±0.02^{Db}	3.65±0.02^{Da}
T_C_1	3.13±0.01^{Be}	3.31±0.03^{Ad}	3.44±0.01^{Ac}	3.68±0.02^{Cb}	3.76±0.02^{Ca}
T_C_2	3.15±0.02^{Be}	3.34±0.02^{Ad}	3.56±0.02^{Ac}	3.87±0.02^{Bb}	3.90±0.02^{Ba}
T_C_4	3.18±0.01^{Ac}	3.62±0.11^{Bb}	3.63±0.41^{Ab}	4.23±0.02^{Aa}	4.39±0.02^{Aa}
Nitrogen content					
T_C	4.28±0.06^{Aa}	4.63±0.31^{Aa}	4.14±0.46^{Aa}	4.24±0.38^{Aa}	4.13±0.36^{Aa}
T_C_1	3.91±0.17^{Aa}	4.33±0.47^{Aa}	4.00±0.31^{Aa}	3.96±0.58^{Aa}	4.15±0.52^{Aa}
T_C_2	4.47±0.52^{Aa}	4.47±0.38^{Aa}	4.31±0.38^{Aa}	4.10±0.48^{Aa}	4.30±0.46^{Aa}
T_C_4	4.35±0.50^{Aa}	4.40±0.37^{Aa}	4.39±0.47^{Aa}	4.19±0.54^{Aa}	4.37±0.45^{Aa}
Protein content					
T_C	27.27±0.39^{Aa}	29.57±1.97^{Aa}	26.44±2.92^{Aa}	27.02±2.43^{Aa}	26.35±2.29^{Aa}
T_C_1	24.95±1.06^{Aa}	27.60±2.98^{Aa}	25.52±1.95^{Aa}	25.26±3.69^{Aa}	26.48±3.30^{Aa}
T_C_2	28.50±3.30^{Aa}	28.54±2.40^{Aa}	27.48±2.44^{Aa}	26.15±3.09^{Aa}	27.43±2.94^{Aa}
T_C_4	27.77±3.19^{Aa}	28.05±2.36^{Aa}	28.01±3.00^{Aa}	26.73±3.45^{Aa}	27.89±2.88^{Aa}
Free fatty acids					
T_C	7.15±0.02^{De}	8.08±0.02^{Dd}	8.33±0.02^{Dc}	8.73±0.02^{Db}	9.05±0.01^{Da}
T_C_1	7.24±0.02^{Ce}	8.21±0.01^{Cd}	8.54±0.02^{Cc}	8.80±0.02^{Cb}	9.10±0.02^{Ca}
T_C_2	7.32±0.01^{Be}	8.27±0.02^{Bd}	8.61±0.01^{Bc}	8.89±0.01^{Bb}	9.15±0.02^{Ba}
T_C_4	7.38±0.03^{Ae}	8.46±0.02^{Ad}	8.90±0.03^{Ac}	9.03±0.02^{Ab}	9.21±0.02^{Aa}
Ripening index					
T_C	7.45±0.23^{Dd}	9.61±0.65^{Dc}	11.59±0.75^{Db}	13.79±1.03^{Da}	14.89±0.75^{Da}
T_C_1	9.66±0.10^{Ce}	12.47±0.98^{Cd}	13.71±0.20^{Cc}	15.38±0.25^{Cb}	16.88±0.24^{Ca}
T_C_2	11.91±0.23^{Bd}	14.53±0.96^{Bc}	17.16±1.73^{Bab}	18.92±1.41^{Ba}	19.75±1.29^{Ba}
T_C_4	13.92±0.24^{Ae}	15.78±0.30^{Ad}	18.99±0.81^{Ac}	20.68±0.83^{Ab}	21.99±0.73^{Aa}

Values are expressed as means±standard deviation (SD)
A-D: Values with different superscripts in the same column are significantly different (p<0.05)
a-e: Values with different superscripts in the same line are significantly different (p<0.05).

and T_C_4 ($p < 0.05$). Since the fat content of caper berries is exceptionally low, the fat content was found to be lower in the caper containing groups. Among all groups, the control group (T_C) had the highest fat in dry matter ratio. The changes of fat in dry matter content of this group throughout ripening was significant ($p < 0.05$). Because the dry matter content of cheese changes throughout ripening, the composition of the cheese may also vary. Consequently, components such as fat and salt were assessed based on their proportions within the dry matter. Also, factors such as proteolytic activity of flora may affect fat content of cheese throughout ripening. Similar results have been reported (Yerlikaya & Karagozlu, 2014).

Evaluation in terms of tyrosine content showed a constant increase in all groups. Particularly, in group T_C, T_C_1 and T_C_2 the increase throughout ripening was found to be significant ($p<0.05$). Alterations regarding the increase in the tyrosine content are considered indicators of proteolysis and similar findings have been stated in other studies (Güven & Karaca, 2001; Ozer et al., 2002; Yerlikaya & Karagozlu, 2014).

The highest nitrogen content of all groups was observed on the 30th day. A significant relationship in the changes of nitrogen content throughout ripening was found in group T_C ($p < 0.05$). Among caper cheese groups, T_C_1 had the lowest nitrogen content (3.91%) on the 1st day. The lowest protein content was found on the 60th day (T_C_2), while the highest was found on the 30th day (T_C). A significant relationship was found between all ripening days in groups T_C and T_C_2 ($p < 0.05$). While there was no significant difference between the 45th and 60th days of storage ($p < 0.05$), the difference between other ripening days was found to be significant in group T_C_1 ($p < 0.05$). In conclusion it can be said that beginning from the 45th day, protein content increased as the caper content increased, but this increase was found to be statistically insignificant ($p > 0.05$). Protein proportions in dry matter did not vary significantly throughout stages of development or between groups ($p > 0.05$). The lowest protein ratio in dry matter of Tulum cheeses with caper was determined on the 60th day and the lowest protein ratio in the dry matter of the control group (T_C) was determined on the 45th day.

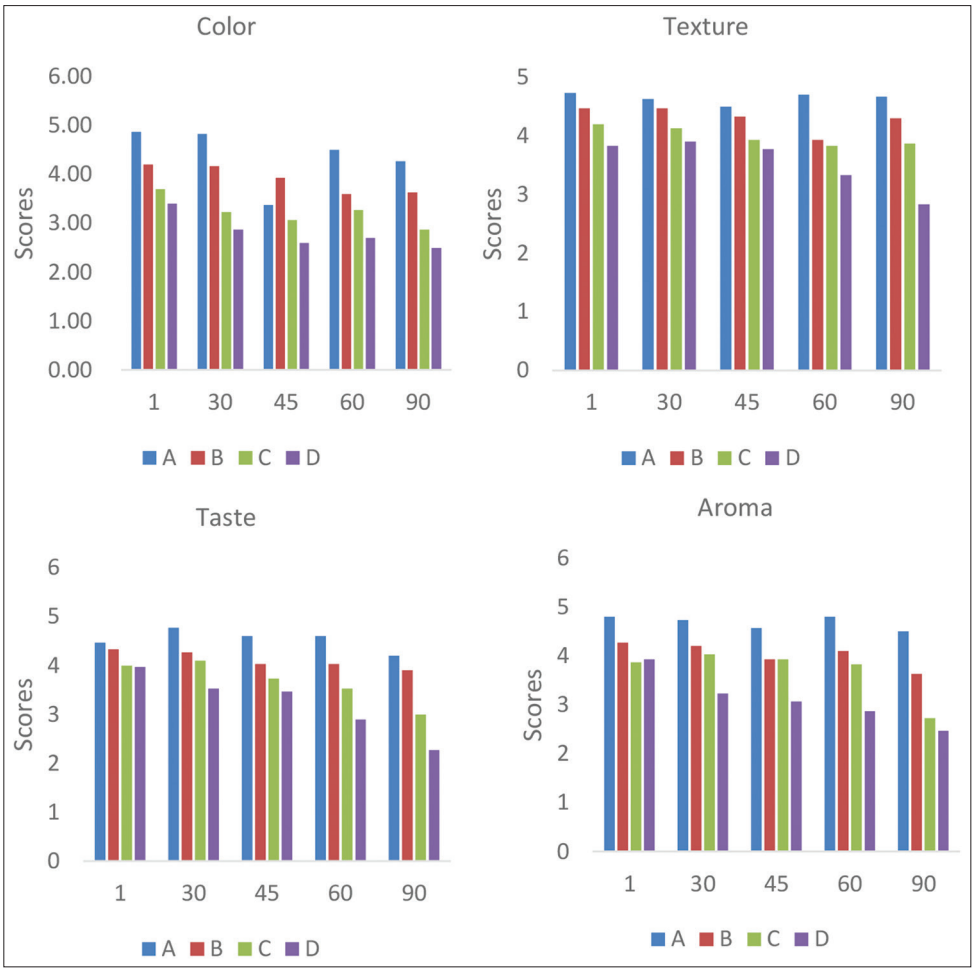

Fig 1. Comparison of changes in sensorial parameters of Tulum cheese during ripening period

The evaluation of free fatty acids revealed that the lowest content was recorded on the first day (group T_C) and the highest value on the last day (T_C_4). In general, in terms of free fatty acids, the control group had the lowest content while group T_C_4 had the highest. A significant relationship was found between the storage days of all groups ($p < 0.05$). It was observed that with the increase in caper content, free fatty acids content also increased.

There was a notable dissimilarity in terms of ripening index between all groups ($p < 0.05$). The lowest and highest RI value were determined on the first day (T_C) and last day of ripening (T_C_4), respectively. It was observed that the RI increased significantly as the caper content increased.

Sensorial results

The highest scores in the sensory evaluation for aroma were given to group T_C, meanwhile group T_C_4 received the lowest. Regarding taste, control group received the highest scores. Aroma scores in caper containing groups decreased during ripening. Among caper containing groups, T_C_1 got the closest scores to the control group (Fig. 1). As the concentration of the caper increased, the bright ceramic colour of the cheese turned towards the dark green colour. The lack of homogeneous distribution of caper affected the structure scores. In general, it was observed that the control group received the highest scores. A significant relationship was found between groups T_C and T_C_2, T_C and T_C_4, and T_C_1 and T_C_4 groups ($p<0.05$). The glucosides present in the caper composition may be responsible for the aroma present in caper containing groups (Çalış et al., 2002). The degradation products of glycosylates present in the composition of caper being dissolved in oil created a tangy odour and taste which negatively affected the taste scores.

Textural results

The textural analysis results are presented in Fig. 2. In terms of hardness, the lowest and highest scores were

Fig 2. Comparison of changes in textural parameters of Tulum cheese during ripening period

given to group T_C and T_C_2, respectively. All groups showed a decrease in scores throughout ripening. Cheese samples containing caper were found to have elevated hardness scores compared to the control cheeses. Salt content and hardness values increased parallelly with each other in Tulum cheese samples. The salt levels of the cheese influence its hardness. The hardness of cheese also increases when the salt level increases (Kaya, 2002). In the first 60 days, springiness scores decreased in all groups. Among caper containing groups, T_C_4 received the highest scores. It can be said that the current structure of the caper berries affected the flexibility of the cheese. Meantime, group T_C received the highest values among all groups. Initially, the lowest resilience scores corresponded to group T_C and the highest to group T_C_1. While the scores of group T_C_1 were higher than the other samples with caper on the 1st and 30th days, it was observed that they got close values on the following days.

A prominent variability was observed between the chewiness scores of all groups ($p < 0.05$). In the obtained results, the lowest chewiness score was observed on the 90th day in group T_C_4 and the highest value on the first day in group T_C_2. There was a decrease in chewiness scores during ripening in all groups. The fact that the caper is harder than other spices and herbs due to their structure has changed their chewiness values.

Generally, the external adhesiveness scores decreased throughout ripening. The lowest external adhesiveness score was found on the 90th day (group T_C_1), and the highest scores was on the 45th day (group T_C_2). Among the capered groups, the highest external adhesiveness score was found in group T_C_2. The internal cohesiveness scores increased during ripening. The lowest internal cohesiveness score was found on the first day belonging to group T_C_4, and the highest scores was found on the 90th day belonging to group T_C_2. It was observed that the gumminess scores decreased throughout ripening in all groups. The control group had the lowest gumminess scores, while group T_C_2 had the highest values. A statistically significant difference was found between the ripening days of the control group ($p < 0.05$).

CONCLUSION

Tulum cheese is produced and consumed in different ways in various regions of Turkey. Various herbs grown in different locations are used in Tulum cheese for purposes such as increasing health benefits, adding a pleasant smell and expanding the varieties of cheeses. Among a diversity of herbs, the antioxidant, antimicrobial, and health effects of *C. spinosa* grown in the Mediterranean region are well-known. The objective of the current investigation was to formulate a probable functional product with additional beneficial effect on general health. The data obtained on the antioxidative properties of caper used in the production of Tulum cheese have been promising in terms of positive effects on consumer health. In addition, the fact that caper do not affect the physicochemical properties of the product negatively is one of the important results of the study. Although the microbial changes occurred at generally acceptable levels during the ripening process, the high number of yeast and mold also reveals that solutions should be provided to reduce the contamination that may especially arise from the drying stage of the production. At the same time, further studies that will reveal the high antioxidative values of caper in the ripening process of Tulum cheese will make the data obtained in this study more meaningful. Generally, when all the properties of the product and obtained results are evaluated, we recommend adding low amounts of caper to Tulum cheese.

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Authors' contribution

In this study, Ahmet Hulusi Dinçoğlu contributed in the processing, experimental work, interpretation of results and manuscript editing. Ali İleri performed experimental analyses and manuscript writing. Jerina Rugji was joined in experimental work and manuscript editing.

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