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ORIGINAL ARTICLE

Some microbiological, physicochemical and ripening properties of Erzincan Tulum cheese produced with added black cumin (*Nigella sativa* L.)

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Abstract The effects of black cumin (BC) at 0, 1 or 2% in Erzincan Tulum cheese (Tulum cheese) on its microbiological, chemical and proteolysis properties during ripening were investigated. The addition of BC caused an increase in the dry matter, protein, ash, total nitrogen (TN), water-soluble nitrogen (WSN) (% of TN) (ripening index), 5% phosphotungstic acid soluble nitrogen (PTA-SN) (% of TN), and 12% trichloroacetic acid soluble nitrogen (TCA-SN) (% of TN) concentrations compared to BC-free (control) cheese. Additionally, the addition of BC caused a decrease in coliforms, yeasts & moulds, fat-in-dry matter (FDM), pH, and acidity. The ripening period had an increasing effect on the dry matter, protein, FDM, salt, salt-in-dry matter (SDM), ash, acidity, TN, WSN, PTA-SN and TCA-SN concentrations. In addition, the ripening period had a decreasing effect on coliforms, yeasts & moulds, and pH. The count of *Staphylococcus aureus* in all Tulum cheeses made of raw sheep's milk was < 2 log cfu/g, ripening was faster and favourable Tulum cheese was produced. Therefore, 2 months after adding 2% BC to Tulum cheese that was produced using raw sheep milk immediately after milking as the original production method, it was found that Tulum cheese can be consumed more safely than coliform bacteria and yeasts & moulds.

Keywords Tulum cheese · Black cumin · *Nigella sativa* L. · Antimicrobial effect · Ripening

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Introduction

There are more than 100 varieties of cheese in Turkey, but three of these varieties (Beyaz, Kaşar and Tulum cheeses, especially Erzincan Tulum- Şavak cheese) are the most popular. Erzincan Tulum cheese (Tulum cheese) has a white or cream colour, high fat content, buttery flavour and semi-hard texture and crumbles easily (Çakmakçı 2011). It received a geographical indication status by the Turkish Patent Institute (TPI 2000) in 2000, and it is the first registered cheese by the TPI in Turkey. According to this document, Tulum cheese is produced in plateaus around Erzincan province using sheep milked between May and September. In comparison to other types of cheeses, the ripening process of Tulum cheese lasts longer. Tulum cheese is produced on the mountains and in plateaus of Erzincan, Erzurum, Bingöl, Tunceli and Elazığ by the Şavak tribe, and it was originally ripened in Tulum caves for 3 months or more. The manufacturing and ripening processes have been reviewed and discussed by Hayaloglu et al. (2007), Çakmakçı (2011) and Cakir et al. (2016) in detail. Erzincan Tulum cheese, which is the best known among the Tulum cheeses, is the third most commonly produced cheese in Turkey. In addition to be convenient for exportation, having high nutritional value and being more expensive than butter increase the economic importance of Tulum cheese.

Black cumin (*Nigella sativa* L.) has been used for more than 2000 years (Salem 2005). Its antibacterial, antifungal, antioxidant, antidiabetic, gastroprotective, anti-inflammatory, anticancer, antihypertensive, and immune-enhancing effects are well-known (Ramadan 2007). Black cumin includes important fatty acids, minerals, vitamins and volatile components. *N. sativa* has nourishing, flavour, adornment and texture features. Therefore, it has been used in various areas. Black cumin also has a therapeutic effect on some diseases (Salem 2005; Çakmakçı and Çakır 2011; Cakir et al. 2016).

Studies on Tulum cheese have focused on their chemical and microbiological characteristics and on the effects of different packaging materials on the various properties of Tulum cheese during ripening (Çakmakçı 2011). Tarakci et al. (2005) studied the effect of black cumin on ripening of Tulum cheese made using cow's milk and the changes that occurred during ripening. Hayaloglu et al. (2007) studied the microbiology, biochemistry and volatile profiles of Tulum cheese, and Cakmakci et al. (2011) studied the proteolysis, sensory properties and gross composition of Tulum cheese ripened in goat's skin or plastic bags.

Due to the many beneficial effects of black cumin, we believed that black cumin can be used as a natural additive in Tulum cheese production. The cheese is made from raw Akkaraman sheep's milk. Akkaraman sheep's milk also has very high fat and protein content. Thus, the cheese has a high economic value and brings higher profits than the other important Turkish cheeses (Beyaz and Kasar cheeses) (Çakmakçı 2011). The effects of the addition of black cumin at levels 0% (control) and 1% (w/w) to Tulum cheese on its sensory properties and volatile profiles have been published before (Cakir et al. 2016). Therefore, the aim of this study was to investigate the possibility of using black cumin during the production of Tulum cheese and to study the antimicrobial properties and effect of black cumin on the quality characteristics of the cheese. The effects of the addition of black cumin at levels of 0% (control), 1 or 2% (w/w) to Tulum cheese on its gross chemical and microbiological composition as well as the ripening characteristics of the Tulum cheese were investigated.

Materials and methods

Materials

Raw Akkaraman sheep's milk (pH 6.91, titratable acidity 0.21%, specific density 1.035, ash 0.97%, protein 5.44%, fat 9.05%, and total solids 20.36%) was used to manufacture Tulum cheese. Raw Akkaraman sheep's milk was obtained from Şavak tribe landing Çat plateaus (Erzurum, Turkey). To coagulate the milk, a homemade rennet (1:1500 MCU/mL) which was obtained from Tulum cheese manufacturers on the plateau was used. Black cumin was obtained in Kahramanmaraş, Turkey. Plastic pots (hardened cylindrical barrels with a 2 kg capacity) used in the packaging of Tulum cheese were supplied from local markets (Elazığ, Turkey).

Cheese-making

Tulum cheese production is shown in Fig. 1.

Homemade calf rennet was added at 35 °C to raw sheep's milk, and coagulation took place after 60 min. The coagulum was processed by the method described in Hayaloglu et al. (2007). Before pressing the curd into hardened plastic barrels, the curd was divided into three parts: black cumin-free (C), black cumin-added at a level of 1% (w/w) (BC1) and black cumin-added at a level of 2% (w/w) (BC2). Preliminary experiments show that when 2% excess black cumin is added to Tulum cheese, the colour of the cheese is blackened and due to the intensity of the black cumin flavour, Tulum cheese has been found to be devoid of flavour characteristics. Curds containing cumin were mixed uniformly and were tightly filled into the hardened barrels. Two Tulum cheese making trials were performed, and the cheeses were ripened for 90 days at 4 ± 1 °C.

Microbiological analysis

For microbiological analysis, 10 g of Tulum cheese was weighed and dispersed aseptically in 90 mL of saline peptone water that included 0.85% NaCl plus 0.1% peptone. The mixture was homogenized (8 strokes per second) in a sterile polyethylene bag using a Stomacher (Mayo

Tulum cheese was made from raw Akkaraman sheep's milk in Çat plateau (Erzurum, Turkey).
↓
The homemade calf rennet was added at a level of 1.0% (vol/vol) at 35 °C into raw sheep's milk.
↓
After approximately 60 min, the coagulum was cut into pieces (~1 cm cubes) and transferred into cotton bags for whey drainage.
↓
Drainage was carried out at ~20 °C for 24 h, and the curd was pressed by piling the cotton bags on top of each other, with regular turning.
↓
Stacking of the curd at this temperature allows curd acidity (pH drops below 6.0) to develop and increases the removal of whey.
↓
Following this step, the curd was broken into pea-size pieces by hand and salted (3.0%, wt/wt), kneaded, and transferred into the bags.
↓
The bags were piled on top of each other and turned regularly at 15-18°C for 10 d.
↓
Afterward, the addition of *N. sativa* (0%, 1% and 2%, wt/wt) by hand uniformly mixing. The plastic containers have drain holes in the cover.
↓
The curds containing with cumin at different levels [0% (C), 1% (BC1) and 2% (BC2)] were tightly filled into the hardened plastic barrels and the packaged samples were ripened at 4±1°C for 90 days.
↓
Cheese making was made in duplicate, and the cheese samples were analysed for every 30 d (1, 30, 60 and 90 days of ripening period).

Fig. 1 Tulum cheese production flow chart

HG400 Stomacher, Italy) for 2 min. Serial dilutions were made in saline peptone water (ISO 6887) (Harrigan 1998), and all tests were performed in duplicate. Total aerobic mesophilic bacteria (TAMB) were enumerated on Plate Count Agar (Merck, Darmstadt, Germany) at 30–32 °C for 48 h (Harrigan 1998), lactobacilli on MRS agar (de Man Rogosa Sharpe Agar, Merck, Darmstadt, Germany) at 30 °C for 3 days under anaerobic conditions (Harrigan 1998), lactococci on M17 agar (Merck, Darmstadt, Germany) at 30 ± 1 °C for 24 or 48 h (Gilliland et al. 1984), total coliforms (Violet Red Bile Agar; Merck, Germany) at 35–37 °C for 48 h (Harrigan 1998), yeasts & moulds on Potato Dextrose Agar (Merck, Darmstadt, Germany) at 25 °C for 5–7 days (Harrigan 1998) and *Staphylococcus aureus* on Baird-Parker Agar (Merck) at 37 °C for 24 h (Harrigan 1998).

Gross chemical composition and proteolysis

The cheese samples were analysed during the ripening period at the 1st, 30th, 60th, and 90th days. Dry matter was investigated by the oven-drying method at 105 °C (IDF 1982), fat content by the method of Gerber, and ash and salt contents according to the methods described by Kurt et al. (2007). Titratable acidity (lactic acid, %) was measured as suggested by AOAC (1995). To measure pH, a grated Tulum cheese sample (10 g) was mixed uniformly with distilled water (15 mL), and the pH of the resultant slurry was measured using a digital pH meter (pH 211, Microprocessor pH Meter, Hanna Inst., Italy). Tulum cheese samples were investigated in duplicate to determinate total nitrogen (TN) by the micro-Kjeldahl method (IDF 1993), and the total protein content was calculated (TN % × 6.38). Water-soluble nitrogen (WSN) (% of TN) in Tulum cheese samples were determined as described by Kuchroo and Fox (1982). Twelve percent trichloroacetic acid-soluble nitrogen (TCA-SN) (% of TN) and 5% phosphotungstic acid-soluble nitrogen (PTA-SN) (% of TN) fractions were obtained by the methods described in Hayaloglu et al. (2007).

Statistical analysis

A randomized complete block design that incorporated 3 treatments (Tulum cheeses: C, BC1 and BC2), 4 ripening periods (1, 30, 60 and 90 days) and 2 trials was used to analyse the relationship of the response variable to the cheese composition data. Analysis of variance was performed using the SPSS package version 9.0 for Windows (SPSS, Inc., Chicago, IL, USA), whereby the effects of the treatments and replicates were estimated for response variables. Duncan's multiple comparison test was used as a guide for paired comparisons of the treatment means using the SPSS package version 9.0 for Windows (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered to be a significant difference between treatments.

Results and discussion

Microbiological characteristics

The microbiological properties of the cheese samples are presented in Table 1. The TAMB, lactobacilli and lactococci counts of the samples showed a continuous decrease until 60 days of ripening. This decrease may be due to the increasing salt and acid concentrations during the ripening period. However, these counts increased at the end of ripening (90 days). These changes were statistically significant during the ripening period ($p < 0.05$) (Table 1). These results agree with those reported by Seratlic et al. (2011) for Gorgonzola cheese. The use of black cumin in the manufacturing of Tulum cheese had no effect on the TAMB, lactobacilli or lactococci counts. In Tulum cheese samples, the highest levels of TAMB, lactobacilli and lactococci counts were found in the first stage of ripening (Table 1). Additionally, the highest and lowest levels of TAMB, lactobacilli and lactococci counts ranged from 8.09 to 6.73, from 8.24 to 6.70 and from 8.03 to 6.72 log cfu/g, respectively (Table 1). It is not surprising that high microbiological counts were found with traditional manufacturing methods due to the use of unpasteurized milk (Ceylan et al. 2007). The results obtained for the TAMB and lactococci counts in this study were lower than those reported by Seratlic et al. (2011) for Gorgonzola cheeses. The results obtained for the TAMB counts in this study were lower than those reported by Hayaloglu and Kirbag (2007) for Kuflu cheese. The results obtained for the lactobacilli counts in this study were higher than those reported by Seratlic et al. (2011).

The total coliform and yeasts & moulds counts of the cheese samples continuously decreased until the end of ripening. This decrease had a significant effect on the total coliform and yeasts & moulds counts of Tulum cheese ($p < 0.05$). A decrease in the total coliform and yeasts & moulds counts during the ripening period has also been reported by Tarakci et al. (2005) for Tulum cheese. Generally, the numbers of coliform bacteria in all samples were high at the beginning of the ripening period. The total coliform and yeasts & moulds counts in the cheese samples decreased with increasing black cumin concentrations ($p < 0.05$) (Table 1). The decrease in pH with the increasing concentration of black cumin may have caused the decrease in the number of coliform bacteria. This decrease was statistically significant ($p < 0.05$) (Table 1). There is no provision on the number of coliform bacteria,

Table 1 The changes in microbiological characteristics of Tulum cheeses during ripening (log cfu/g)

Type of cheese (treatments)	Ripening time (days)	TAMB*	Lactobacilli	Lactococci	Total coliforms	Yeasts & moulds	*S. aureus*
C (0% bc)	1	8.09 ± 0.50	8.06 ± 0.20	8.03 ± 0.01	3.73 ± 0.91	6.14 ± 0.35	< 2
	30	6.97 ± 0.15	7.51 ± 0.23	6.90 ± 0.07	3.21 ± 0.26	4.90 ± 0.13	< 2
	60	6.99 ± 0.08	6.84 ± 0.16	6.94 ± 0.08	2.71 ± 0.40	4.41 ± 0.08	< 2
	90	7.19 ± 0.08	7.34 ± 0.06	7.14 ± 0.01	2.15 ± 0.21	4.13 ± 0.03	< 2
	Average	7.31 ± 0.50a	7.44 ± 0.48a	7.25 ± 0.49a	2.95 ± 0.74a	4.90 ± 0.84a	< 2
BC1 (1% bc)	1	8.09 ± 0.01	8.18 ± 0.02	7.93 ± 0.25	3.03 ± 0.16	5.77 ± 0.23	< 2
	30	7.10 ± 0.23	7.90 ± 0.15	7.28 ± 0.61	2.40 ± 0.45	4.71 ± 0.11	< 2
	60	6.99 ± 0.13	6.85 ± 0.04	6.72 ± 0.03	2.02 ± 0.06	4.39 ± 0.28	< 2
	90	7.13 ± 0.31	7.62 ± 0.03	7.21 ± 0.16	1.60 ± 0.00	4.02 ± 0.03	< 2
	Average	7.33 ± 0.50a	7.64 ± 0.53a	7.28 ± 0.53a	2.26 ± 0.59b	4.72 ± 0.71ab	< 2
BC2 (2% bc)	1	7.90 ± 0.14	8.24 ± 0.41	7.71 ± 0.01	2.29 ± 0.01	5.67 ± 0.11	< 2
	30	7.55 ± 0.12	7.72 ± 0.42	7.19 ± 0.01	1.80 ± 0.02	4.82 ± 0.17	< 2
	60	6.78 ± 0.02	6.70 ± 0.17	6.78 ± 0.18	< 1	4.04 ± 0.13	< 2
	90	6.73 ± 0.30	7.18 ± 0.08	6.85 ± 0.18	< 1	3.59 ± 0.27	< 2
	Average	7.24 ± 0.55a	7.46 ± 0.66a	7.13 ± 0.40a	1.02 ± 1.11c	4.53 ± 0.86b	< 2
Treatment average	1	8.02 ± 0.12a	8.16 ± 0.22a	7.89 ± 0.13a	3.01 ± 0.77a	5.86 ± 0.30a	–
	30	7.20 ± 0.30b	7.71 ± 0.28b	7.12 ± 0.33b	2.47 ± 0.68b	4.81 ± 0.14b	–
	60	6.92 ± 0.13c	6.80 ± 0.13c	6.81 ± 0.13c	1.58 ± 1.27c	4.28 ± 0.24c	–
	90	7.01 ± 0.30bc	7.38 ± 0.21d	7.07 ± 0.20bc	1.25 ± 1.00c	3.91 ± 0.28d	–

C: Tulum cheese (0%, Control); BC1: 1% the black cumin-added Tulum cheese; BC2: 2% the black cumin-added Tulum cheese; bc: black cumin

Averages of the same column values (each section separately) by the same letter did not differ significantly from Duncan's multiple range tests at 5% significance

(a–d) Mean ± SD, values followed by the same letters within a column are significantly different at $p < 0.05$

yeast & moulds in Tulum Cheese Standard (TS 3001) (TS-3001 2016) and in Turkish Food Codex Regulation–Microbiological Criteria Communiqué (2009/68) regarding Tulum cheese. However, it has been reported that the Enterobacteriaceae family can have a maximum of 10^3 cfu/g. According to the Food Safety Center (2014), the number of entrances of ripened cheeses can be accepted in the range of 10^2–10^4 cfu/g. The number of *Escherichia coli* can be up to 10^2 cfu/g, but the decision does not apply to cheeses made from raw milk. According to Scientific Criteria to Ensure Safe Food (2003), cheeses made from raw milk and from thermified milk (Directive 92/46/EEC) indicate that up to 10^4 *E. coli* can be found.

In this study, black cumin had antimicrobial activity. Therefore, black cumin was added to decrease the total coliform counts and yeasts & moulds in Tulum cheese samples. The increasing concentration of black cumin causes the decrease in the number of coliform bacteria and yeasts & moulds (Table 1). The BC2 samples had lower total coliforms and yeasts & moulds counts than the BC1 and C (control) samples. The BC1 samples also had lower total coliform and yeasts & moulds counts than the C samples. In Tulum cheese samples, the highest and lowest levels of total coliforms and yeasts & moulds were noted for C Tulum cheeses at 1 day of ripening and BC2 Tulum cheeses at 90 days of ripening, respectively (Table 1). The total coliform and yeasts & moulds counts in the Tulum cheese samples during ripening ranged from < 1–3.73 to 3.59–6.14 log cfu/g (Table 1). In this study, the yeasts & moulds counts were lower than those reported by Cakmakci et al. (2012) for mouldy Civil cheese and Hayaloglu and Kirbag (2007) for Kuflu cheese. The results obtained for total coliforms were in agreement with the results reported by Cakmakci et al. (2012) for mouldy Civil cheese and Hayaloglu and Kirbag (2007) for Kuflu cheese.

The addition of black cumin and the ripening period decreased the total coliform and yeasts & moulds counts in the cheese samples (Table 1). Because black cumin had antimicrobial activity (Salem 2005; Çakmakçı and Çakır 2011). In general, the total coliform counts are higher in Tulum cheese compared to other types of cheeses because raw milk is used during the production of Tulum cheese. In this study, therefore, black cumin was added to decrease the total coliform counts and yeasts & moulds in Tulum cheese samples. The counts of total coliforms and yeasts & moulds reported in Tulum cheese samples were lower than those reported by Hayaloglu et al. (2007). In this study, the *S. aureus* counts were < 2 log cfu/g in all Tulum cheese samples (Table 1). *S. aureus* was not detected in any samples during ripening (Table 1). According to the TS 3001 (2016) and the Turkish Food Codex Regulation–Communiqué on Microbiological Criteria (2009/68), the number of *S. aureus* of all cheeses except melted cheese can be accepted as 10^2 cfu/g. In this study, we obtained the same *S. aureus* counts as Tarakci et al. (2005) in Tulum cheese. *S. aureus* was found in Kuflu cheese at levels of 2.00–4.92 log cfu/g (Hayaloglu and Kirbag 2007). The storage temperature (2–4 °C) and salt concentration (2.5–3%) may be limiting factors for the growth of these microorganisms.

Chemical characteristics

The mean and standard deviation for the dry matter, fat-in-dry matter (FDM), total protein, ash, salt, salt-in-dry matter (SDM), pH and titratable acidity of Tulum cheese samples during ripening are listed in Table 2. The addition of black cumin and the ripening period increased dry matter, ash and total protein. The dry matter contents ranged from 60.78 to 62.62% in the samples, and the values increased during the ripening period and with increasing black cumin levels ($p < 0.05$) (Table 2). The increases in the physico-chemical parameters were due to a decrease in moisture during ripening. The decrease in moisture is caused by microbial multiplication and the resulting acid development that occurs during ripening, as well as by syneresis of the cheeses. According to the TS-3001 (2016), the moisture content of the Tulum cheese is limited to at most 45%. Other contributing factors may be the reduced hydration of casein as the pH reaches the isoelectric point or casein as well as the evaporation of moisture from cheese through the pores of the casing material (Fox et al. 1999; Ceylan et al. 2007).

In this study, the dry matter values of the cheese samples were higher than those reported by Cakmakci et al. (2011) for Tulum cheeses, Galli et al. (2016) for Camembert-type cheese, and Hayaloglu and Kirbag (2007) for Kuflu cheese. The total protein values of the cheese samples were higher than those reported by Hayaloglu et al. (2007) for Tulum cheese. The total protein values of cheese samples were lower than those reported by Hayaloglu and Kirbag (2007) and Hayaloglu et al. (2008) for Kuflu cheeses. The ash values of the cheese samples were lower than those reported by Sert et al. (2014) for Tulum cheese. In the cheese samples, the highest and lowest values of dry matter, ash and total protein were noted for the BC2 Tulum cheeses at 90 days of ripening and C Tulum cheeses at 1 day of ripening, respectively (Table 2). The increased dry matter content of the BC1 and BC2 cheeses was noticeable and occurred because they were produced with black cumin, resulting in a higher dry matter (98%) content than black cumin-free (C) cheese. Seeds can cause very small spaces, which may result in increased syneresis. In addition, the increase in dry matter, salt, fat and ash amounts can be attributed to the relative increase in these values due to loss of moisture in the cheese. The moisture level was below 45%, as described in the regulations for Tulum cheese according to the Turkish Food Codex (Anonymous 2015). The dry matter was not below 60%, as is specified in the regulations for Tulum cheese in the Turkish Food Codex (Anonymous 2015). In this study, the dry matter values of the cheese samples, which were higher than those reported by Hayaloglu et al. (2007), Cakmakci et al. (2011) and Sert et al. (2014) for Tulum cheeses, Hayaloglu and Kirbag (2007) for Kuflu cheese were acceptable.

The FDM contents of the Tulum cheese samples increased until the 30th day of ripening ($p < 0.05$) and slightly increased from the 30th day to the 90th day of ripening. The decreased level of FDM in the BC1 and BC2 cheeses was noticeable and occurred because the cheese produced with black cumin had a lower fat content than black cumin-free (C) cheese. Increasing the black cumin level in the Tulum cheese samples resulted in a lower FDM content than in black cumin-free (C) samples ($p < 0.05$). The highest and lowest values of FDM were recorded in the C (57.94%, the final value) and BC2 (55.55%, the initial value) Tulum cheeses, respectively (Table 2). These values were higher than those reported by Güven and Konar (1994) and Sert et al. (2014) for Tulum cheese, Hayaloglu and Kirbag (2007) and Hayaloglu et al. (2008) for Kuflu cheese, but the results agreed with the values reported by Hayaloglu et al. (2007) for Tulum cheese. The FDM content of Tulum cheese was ≥ 45% (v/w); therefore, the cheeses could be categorized as full-fat Tulum cheeses according to the regulations for Tulum cheese described by the Turkish Food Codex (Anonymous 2015).

Tulum cheese has very high dry matter, total protein, fat and ash content. The amount of dry matter, total protein and ash of black cumin-added Tulum cheeses were significantly higher than control Tulum cheese (Table 2). Tulum cheese obtained from raw Akkaraman sheep's milk has a very high nutritional values to consumers. In addition, the economic value of this milk is increasing according to the amount of fat that it contains. The addition of black cumin to Tulum cheese made from raw sheep's milk provides a new kind of Tulum cheese because black cumin has antimicrobial effect, nutritional value, a pleasant flavour and has a therapeutic effect on some diseases in addition to including important fatty acids, minerals, vitamins and volatile components (Salem 2005; Çakmakçı and Çakır 2011).

Table 2 The changes in chemical characteristics of Tulum cheeses during ripening

Types of cheese (treatment)	Ripening time (days)	Dry matter (%)	Fat in dry matter (%)	Total protein (%)	Ash (%)	Salt (%)	Salt in dry matter (%)	pH	Acidity (%)
C (0% bc)	1	60.78 ± 0.02	56.97 ± 0.27	21.00 ± 0.01	3.80 ± 0.01	2.64 ± 0.16	4.34 ± 0.27	5.02 ± 0.03	0.85 ± 0.00
	30	61.23 ± 0.03	57.78 ± 0.26	21.04 ± 0.03	3.85 ± 0.01	2.73 ± 0.08	4.46 ± 0.14	4.94 ± 0.04	0.91 ± 0.01
	60	61.67 ± 0.05	57.78 ± 0.33	21.08 ± 0.04	3.95 ± 0.05	2.87 ± 0.08	4.66 ± 0.13	4.82 ± 0.00	1.00 ± 0.06
	90	62.13 ± 0.03	57.94 ± 0.03	21.14 ± 0.01	4.08 ± 0.02	3.11 ± 0.10	5.01 ± 0.16	4.69 ± 0.01	1.05 ± 0.09
	Average	61.45 ± 0.54a	57.62 ± 0.45a	21.06 ± 0.06a	3.92 ± 0.12a	2.84 ± 0.21ab	4.62 ± 0.30a	4.87 ± 0.14a	0.95 ± 0.10a
BC1 (1% bc)	1	60.87 ± 0.02	56.28 ± 0.02	21.45 ± 0.06	3.86 ± 0.01	2.55 ± 0.03	4.19 ± 0.04	4.82 ± 0.00	0.86 ± 0.03
	30	61.34 ± 0.04	56.86 ± 0.25	21.62 ± 0.03	3.93 ± 0.02	2.69 ± 0.00	4.39 ± 0.01	4.81 ± 0.01	0.89 ± 0.04
	60	61.80 ± 0.03	56.84 ± 0.26	21.65 ± 0.02	4.10 ± 0.02	3.10 ± 0.08	5.02 ± 0.13	4.78 ± 0.02	0.91 ± 0.04
	90	62.23 ± 0.03	57.05 ± 0.02	21.71 ± 0.04	4.16 ± 0.01	3.27 ± 0.05	5.25 ± 0.08	4.73 ± 0.01	0.97 ± 0.00
	Average	61.56 ± 0.55b	56.75 ± 0.34b	21.60 ± 0.11b	4.01 ± 0.13b	2.90 ± 0.31b	4.71 ± 0.47a	4.78 ± 0.04b	0.91 ± 0.05b
BC2 (2% bc)	1	61.44 ± 0.08	55.55 ± 0.22	21.93 ± 0.08	3.89 ± 0.00	2.43 ± 0.20	3.96 ± 0.33	4.79 ± 0.01	0.78 ± 0.02
	30	61.79 ± 0.08	56.04 ± 0.21	22.03 ± 0.11	3.95 ± 0.01	2.48 ± 0.20	4.01 ± 0.33	4.77 ± 0.01	0.81 ± 0.01
	60	62.18 ± 0.04	56.29 ± 0.04	22.10 ± 0.04	4.11 ± 0.02	2.81 ± 0.08	4.52 ± 0.14	4.75 ± 0.01	0.86 ± 0.00
	90	62.62 ± 0.05	56.50 ± 0.23	22.16 ± 0.06	4.20 ± 0.01	3.20 ± 0.01	5.11 ± 0.03	4.69 ± 0.01	0.89 ± 0.01
	Average	62.01 ± 0.47c	56.09 ± 0.41c	22.06 ± 0.11c	4.04 ± 0.13c	2.73 ± 0.35a	4.40 ± 0.53b	4.75 ± 0.04c	0.84 ± 0.05c
Treatment Average	1	61.03 ± 0.32a	56.26 ± 0.66a	21.46 ± 0.42a	3.85 ± 0.04a	2.54 ± 0.15a	4.16 ± 0.26a	4.88 ± 0.11a	0.83 ± 0.04a
	30	61.45 ± 0.27b	56.89 ± 0.80b	21.56 ± 0.45b	3.91 ± 0.05b	2.63 ± 0.15a	4.29 ± 0.27a	4.84 ± 0.08b	0.87 ± 0.05a
	60	61.88 ± 0.24c	56.97 ± 0.70b	21.61 ± 0.46bc	4.05 ± 0.08c	2.93 ± 0.15b	4.73 ± 0.25b	4.78 ± 0.03c	0.92 ± 0.07b
	90	62.33 ± 0.23d	57.16 ± 0.66b	21.67 ± 0.46c	4.15 ± 0.06d	3.19 ± 0.09c	5.12 ± 0.14c	4.70 ± 0.02d	0.97 ± 0.08c

C: Tulum cheese (0%, Control); BC1: 1% the black cumin-added Tulum cheese; BC2: 2% the black cumin-added Tulum cheese; bc: black cumin; TAMB: total aerobic mesophilic bacteria

Averages of the same column values (each section separately) by the same letter did not differ significantly from Duncan's multiple range tests at 5% significance

(a–d) Mean ± SD, values followed by the same letters within a column are significantly different at $p < 0.05$

The salt and salt-in-dry matter (SDM) contents of the samples did not increase from the 1st day to the 30th day of ripening, but they significantly increased from the 30th day to the 90th day of ripening ($p < 0.05$). The changes in the salt content of samples after the addition of black cumin were significant ($p < 0.05$). Furthermore, the SDM contents of the C and BC1 cheese samples were not different, but the SDM content of the BC2 cheese sample was different than the other groups ($p < 0.05$). The highest and lowest values of salt and SDM were recorded in BC1 (3.27–5.25%, the final value) and BC2 (2.43–3.96%, the initial value) Tulum cheeses, respectively (Table 2). In this study, the SDM content of the cheese samples was higher than that reported by Cakmakci et al. (2011) for Tulum cheese. The salt and SDM values of the cheese samples were lower than those reported by Sert et al. (2014) for Tulum cheese.

The addition of black cumin decreased the acidity and pH of the samples ($p < 0.05$). The ripening period increased the acidity of the samples but decreased the pH of the samples. The pH of the cheese samples continuously decreased during the ripening period ($p < 0.05$). Normally, a reduction in pH is expected to occur at the initial stages of ripening due to the metabolism of residual lactose to lactic acid, followed by an increase in pH depending on the type of cheese (Fox et al. 1999; Sert et al. 2014). Increasing the black cumin level in the cheese samples resulted in lower pH values than in black cumin-free (C) samples ($p < 0.05$). The highest and lowest pH values were recorded in the C (5.02, the initial value) and C-BC2 (4.69, the final value) cheese samples, respectively (Table 2). The pH of mould-ripened cheeses increases during ripening due to the catabolism of lactic acid and deamination of amino acids by moulds (Hayaloglu et al. 2008). The pH values of the cheese samples were lower than those reported by Hayaloglu and Kirbag (2007) and Hayaloglu et al. (2008) for Kuflu cheeses, Galli et al. (2016) for Camembert-type cheese and Oluk et al. (2014) for Tulum cheese. The pH values of the Tulum cheese samples were lower than the pH values for blue-type cheeses, including commercial Stilton (Madkor et al. 1987) and Kuflu (Hayaloglu et al. 2008) cheese. The high pH in these types of cheeses has been linked with the catabolism of lactic acid in cheese by mycoflora (Cakmakci et al. 2012). The pH values of the cheese samples were in agreement with the results reported by Cakmakci et al. (2011) for Tulum cheese. The acidity content of the cheese samples was not significantly different from the 1st day to the 30th day of ripening but was significantly different from the 30th to the 90th day of ripening ($p < 0.05$). The addition of black cumin had a reducing effect on the acidity of those cheese samples ($p < 0.05$). The highest and lowest values of acidity were recorded in C (1.05%, the final value) and BC2 (0.78%, the initial value), respectively (Table 2). The acidity values of the cheese samples were lower than those reported by Hayaloglu et al. (2007), Ceylan et al. (2007), Cakmakci et al. (2011), Sert et al. (2014) and Oluk et al. (2014) for Tulum cheeses.

The changes in proteolysis values

The mean and standard deviation values for the TN, WSN, TCA-SN and PTA-SN contents of cheese samples during ripening are given in Table 3. The concentrations of TN, WSN, TCA-SN and PTA-SN in the cheese samples increased significantly during ripening (until the end of ripening) ($p < 0.05$). This increase in the concentrations of TN, WSN, TCA-SN and PTA-SN in cheese samples during the ripening period was also reported by Hayaloglu et al. (2007), Tarakci et al. (2005) and Cakmakci et al. (2011) for Tulum cheeses. The addition of black cumin had an effect on the TN, WSN, TCA-SN and PTA-SN contents ($p < 0.05$). The highest and lowest contents of TN, WSN, TCA-SN and PTA-SN were recorded in BC2 Tulum cheese at 90 days of ripening and C Tulum cheese at 1 day of ripening, respectively (Table 3).

The TN measured in the cheese samples was higher than that reported by Cakmakci et al. (2011) for Tulum cheese. The TN measured in the cheese samples was in accordance with that reported by Guler and Uraz (2003) and Güven and Konar (1994) for Tulum cheese.

The WSN determined in Tulum cheese samples was lower than those results reported by Oner et al. (2003), Ceylan et al. (2007) and Cakmakci et al. (2011) for Tulum cheese, and Hayaloglu et al. (2008) for Kuflu cheese. The levels of WSN were in accordance with those reported by Güven and Konar (1994), Guler and Uraz (2003) and Tarakci et al. (2005) for Tulum cheese. The levels of WSN in Tulum cheese samples were lower than those reported for Stilton (Madkor et al. 1987) and Kuflu (Hayaloglu et al. 2008) cheese. The low level of WSN (mean values) in Tulum cheese in comparison with other blue-type cheeses may be linked to the age of the samples, high levels of salt, germination stages of fungi under uncontrolled conditions, among others (Cakmakci et al. 2012).

TCA-SN contains only small peptides with a chain length of between 2 and 20 amino acids, and free amino acids and TCA-SN increase during ripening due to the action of starter or non-starter lactic acid bacteria. The action of lactococci and lactobacilli in cheeses was identically reflected in the concentrations of TCA-SN (Cakmakci et al. 2011). TCA-SN determined in Tulum cheese samples was in accordance with those reported by Cakmakci et al. (2011), Tarakci et al. (2005), Guler and Uraz (2003), Güven and Konar (1994) for Tulum cheeses and was lower than those reported by Hayaloglu et al. (2008) for Kuflu cheese and Oner et al. (2003) for Tulum cheese.

Table 3 The changes in proteolysis levels of Tulum cheeses during ripening

Type of cheese (treatments)	Ripening period (days)	Total nitrogen (TN) %	Ripening index, WSN (% TN)	TCA-SN (% TN)	PTA-SN (% TN)
C (0% bc)	1	3.29 ± 0.00	4.77 ± 0.22	2.67 ± 0.17	1.95 ± 0.17
	30	3.30 ± 0.00	8.10 ± 0.10	5.87 ± 0.29	3.24 ± 0.25
	60	3.30 ± 0.01	13.48 ± 0.16	7.22 ± 0.13	5.24 ± 0.07
	90	3.31 ± 0.00	19.97 ± 0.07	8.14 ± 0.15	7.00 ± 0.17
	Average	3.30 ± 0.01a	11.58 ± 6.15a	5.97 ± 2.22a	4.36 ± 2.06a
BC1 (1% bc)	1	3.36 ± 0.01	4.85 ± 0.07	3.23 ± 0.31	2.01 ± 0.18
	30	3.39 ± 0.01	9.08 ± 0.12	6.55 ± 0.03	3.63 ± 0.08
	60	3.39 ± 0.00	14.56 ± 0.22	7.84 ± 0.16	5.60 ± 0.13
	90	3.40 ± 0.01	21.24 ± 0.31	8.52 ± 0.06	7.31 ± 0.08
	Average	3.39 ± 0.02b	12.43 ± 6.57b	6.54 ± 2.18b	4.64 ± 2.14b
BC2 (2% bc)	1	3.44 ± 0.01	4.89 ± 0.06	3.42 ± 0.09	2.08 ± 0.19
	30	3.45 ± 0.02	9.73 ± 0.40	6.65 ± 0.03	3.91 ± 0.14
	60	3.46 ± 0.01	15.20 ± 0.17	8.11 ± 0.07	6.06 ± 0.14
	90	3.47 ± 0.01	22.00 ± 0.07	8.83 ± 0.20	7.75 ± 0.10
	Average	3.46 ± 0.02c	12.95 ± 6.81c	6.75 ± 2.22c	4.95 ± 2.29c
Treatment average	1	3.36 ± 0.07a	4.84 ± 0.12a	3.11 ± 0.38a	2.01 ± 0.16a
	30	3.38 ± 0.07b	8.97 ± 0.76b	6.36 ± 0.40b	3.60 ± 0.33b
	60	3.39 ± 0.07bc	14.42 ± 0.79c	7.72 ± 0.42c	5.63 ± 0.38c
	90	3.40 ± 0.07c	21.07 ± 0.93d	8.50 ± 0.33d	7.35 ± 0.35d

C: Tulum cheese (0%, Control); BC1: 1% the black cumin-added Tulum cheese; BC2: 2% the black cumin-added Tulum cheese; bc: black cumin

Averages of the same column values (each section separately) by the same letter did not differ significantly from Duncan's multiple range tests at 5% significance

(a–d) Mean ± SD, values followed by the same letters within a column are significantly different at $p < 0.05$

PTA-SN contains small peptides with molecular weights below 600 Da and free amino acids (McSweeney and Fox 1997; Cakmakci et al. 2011). The ripening period had an effect on the formation of PTA-SN in Tulum cheese. It is likely that lactic acid bacteria, which have nutritional requirements for amino acids, are responsible for the formation of PTA-SN in cheese (Macedo and Malcata 1997; Cakmakci et al. 2011). The PTA-SN levels in the Tulum cheese samples were higher than those reported by Cakmakci et al. (2011) and Tarakci et al. (2005) for Tulum cheese and were lower than those reported by Oner et al. (2003) for Tulum cheese. The PTA-SN levels were in accordance with the values determined by Guler and Uraz (2003) and Güven and Konar (1994) for Tulum cheese.

Conclusion

The purpose of this study was to investigate the effects of black cumin (*N. sativa* L.) on the quality characteristics of Tulum cheese during ripening. The addition of black cumin, which has antimicrobial effects, nutritional value and a pleasant flavour, to Tulum cheese obtained from raw Akkaraman sheep's milk resulted in a new Tulum cheese. Depending on the level of black cumin, it was determined that the numbers of coliform bacteria and yeasts & moulds in the cheese decreased. The number of *S. aureus* was < 2 log cfu/g during storage in all samples. In this study, black cumin had antimicrobial activity.

When the research findings are evaluated collectively; in the case of 2% black cumin added to Tulum cheese, it was found that the number of yeasts & moulds provided legal limits after 2 months. Considering the microbiological results, it can be said that in terms of 3 different microorganisms (coliform bacteria, *S. aureus* and yeasts & moulds) cheese can be consumed after 2 months. Depending on the level of black cumin, ripening index was also of the cheese was higher during the ripening period.

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Compliance with ethical standards

Conflict of interest There is no conflict of interest to declare.

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