Small Ruminant Research 167 (2018) 32–38

Contents lists available at ScienceDirect

Small Ruminant Research

journal homepage: www.elsevier.com/locate/smallrumres

Fresh ovine cheese supplemented with saffron (*Crocus sativus* L.): Impact on microbiological, physicochemical, antioxidant, color and sensory characteristics during storage

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ARTICLE INFO

Keywords:
Saffron
Crocus sativus L.
Fresh ovine cheese
Antioxidant activity
Antimicrobial activity
Sensory characteristics

ABSTRACT

Saffron supplemented ovine fresh cheese was studied for its compositional, microbiological, color, antioxidant and sensory characteristics, during cold storage at 4°C for 30 days. The evolution of the total aerobic bacteria and the starter lactococci group, was not remarkably affected, during manufacture, however a significant decrease was observed during storage. In addition, the cheese exhibited a more intensive antimicrobial activity against coliform and enterococci groups, which could be attributed to the saffron presence. Saffron cheese didn't show any remarkable changes in physicochemical properties. However, an enhanced antioxidant activity was observed on 1st day of manufacture and an increasing proteolysis rate was shown after 20 days of storage. Main changes were observed on color and sensory characteristics. The color coordinate b^* was increased with the saffron concentration, suggesting that cheese color gets yellower. The cheese made from milk supplemented with saffron concentration of 50 mg/L was tastefully accepted and brought out traditional sensory characteristics, familiar to Greek consumers.

1. Introduction

Greece is one of the major ovinemilk producers in Europe. Most of ovine milk produced in Greece is used for the manufacture of Protected Designation of Origin (PDO) cheeses. Recently food industry to enrich the list of produced foodstuffs, with high added value and healthy claims for the consumers, has involved in the manufacturing process the addition of different spices, such as paprika, peppercorns and saffron. Saffron is the dried stigma of *Crocus sativus L.*, an indigenous spice found in the Middle East, but also grown in the North Africa, Spain, Austria, France and Greece. *Crocin* and *crocetin*, are the two major natural carotenoids of saffron, responsible for its color (Mohajeri et al., 2010). This spice is a reddish-brown or golden yellow odoriferous powder with slightly bitter taste attributed to *picrococin,* a monoterpene glycoside precursor of safranal T (Ghorpade et al., 1995). *Safranal* is another powerful component which enriches the base product with flavor and aroma (Carmona et al., 2006). The pharmacological effects of aqueous or alcoholic extracts of *C. sativus* stigmas and their biomedical properties as anticonvulsant, antidepressant, anti-inflammatory, antinociceptive and antitumor in humans, have been largely described in the bibliography (Hosseinzadeh et al., 2003; Venkatachalam et al., 2017). Although there are many studies regarding cheese supplementation with herbs and spices, (Carpino et al., 2008) and limited references concerning saffron addition in hard ovine cheeses (Licón et al., 2012), there are no studies at all relevant to the saffron implementation in fresh spreadable ovine cheeses. Fresh cheeses are known to be perishable products due to their high moisture and low salt content ranged from 1 to 1.5% weight by weight (w/w) with short shelf-life, indicating a negative effect on the market system of Small to Medium Enterprises (SME's). In addition, an important safety trait is a possible post-processing contamination and growth of pathogenic microorganisms in fresh cheeses. Given all the above aspects, saffron addition could be used as an attractive alternative practice of improving the flavor and the shelf-life of these kind of cheeses.

Considering these aspects, the objectives of this study were (1) to produce a new fresh spreadable ovine cheese supplemented with saffron extract and (2) to study its main compositional, microbiological, antioxidant and sensory characteristics.

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https://doi.org/10.1016/j.smallrumres.2018.07.016
Received 12 December 2017; Received in revised form 25 April 2018; Accepted 20 July 2018
Available online 29 July 2018
0921-4488/

2. Materials and methods

2.1. Materials

Three successive cheese trials were carried out during the three first weeks of April 2016 in the mid-lactation period. Bulk ovine milk was obtained from the herd (120 sheep) of Animal Science and Aquaculture Faculty of Agricultural University of Athens, Greece. Its composition (% w/w) was determined for each trial by the Milkoscan apparatus (model 255 A/B, type 25700, Fossoelectric, Denmark) and the mean values (±) the standard error (n = 3), for all batches were as follows: 6.10 ± 0.05 fat, 5.20 ± 0.02 total protein, 4.95 ± 0.05 lactose, and 17.15 ± 0.04 dry matter. Finally, the milk ash content (0.95 ± 0.05) was determined according to the reference method (AOAC, 1995). The saffron spice used was the Greek red Saffron «*Krokos Kozanis*» with Protected Designation of Origin (PDO). Starter cultures including the mesophilic strains *Lactococcus lactis subsp lactis* ACA-DC 50 and *Lactococcus lactis* subsp. *cremoris* ACA-DC 47 were obtained from the Culture Collection of Agricultural University of Athens Greece, (ACA-DC). The powder animal rennet was obtained from Chr-Hansen's Laboratories, (Copenhagen, Denmark).

2.2. Saffron extraction

Saffron dried stigmasthreadlike parts of the flower (0.5 g) previously grinded were added in 0.5 L of milk (1000 mg/L) at 42°C, under slow agitation for 45 min. After almost complete extraction the mixture was filtered through a 500 μm mesh sieve and the filtrate, considered as a saffron extract equivalent to 1000 mg/L, was used in the cheese trials.

2.3. Cheese manufacturing

Three fresh cheese making trials were carried out during 3 successive weeks. In each trial, cheeses were made in four different vats. Ovine milk (8 L) was pasteurized at 68 °C for 10 min and distributed in four equal amounts of 2 L each, for the control cheese M and the saffron supplemented cheeses A, B and C respectively. The cheese M was produced with ovine milk without saffron supplementation, while for cheeses A, B and C the milk was supplemented correspondingly with the saffron extract equivalent to 50, 75 and 100 mg/L saffron concentration. After pasteurization, the milk was cooled at 30 °C and each milk vat was inoculated with starter culture containing about 10^8 cfu/mL at the rate of 1% (3.5×10^6 cfu/g). After an hour pre-incubation at 30 °C, 100, 150 and 200 mL of the concentrated saffron extract (1000 mg/L), were added in each of the vats A, B and C, respectively. Commercial rennet (0.25 g) and salt 1% weight by volume (w/v), were added under stirring in all vats. The mixtures were incubated at 25–28 °C for about 12 h, until the curd reached the pH value of 4.6. The curds were set in cheese-cloths and were hanged in a ripen chamber for draining at 25 °C for 6 h until pH value was decreased to 4.4. After drainage, the soft cheeses were distributed in plastic cups of 100 g and were stored at 4 °C, for sampling and analyzing in duplicate on 1, 10, 20 and 30 days.

2.4. Microbiological analysis

The pasteurized milk for each cheese-trial was analyzed for total bacterial count (TBC), while the microbial profile of the saffron-extract was analyzed by conventional microbiological methods for more bacteria groups. To study the microbiological profile of cheeses throughout cold storage, selective groups of microorganisms were enumerated. Ten grams of cheese sample was added to 90 mL of sterile (w/w) sodium citrate buffer and stomached for 4 min at 260 rpm using a Stomacher 400 (Seward Laboratory Systems). Serial 10-fold dilutions in sterile Ringer solution were prepared and seeded in the corresponding culture medium in duplicate. All results were expressed as colony forming units per gram of cheese (cfu/g) (ISO 4833-1, 2003). TBC were enumerated on plate count agar (PCA; Biokar Diagnostics, Beauvais, France) after incubation at 30 °C for 72 h aerobically. Lactococci counts were enumerated in M17 agar (Biokar Diagnostics, Barcelona, Spain) after incubation at 30 °C for 48 h aerobically. For enterococci counts Kanamycin Esculin Azide agar was used (KAA; Merck, Darmstadt, Germany) at 37 °C for 24 h. Violet Red Bile Lactose agar was used for coliforms (VRBL; Biokar Diagnostics, Barcelona, Spain) at 37 °C for 24 h. Micrococci were grown in Mannitol Salt agar (MSA; LAB-M, Neogen Company, Lancashire, UK) incubated at 30 °C for 48 h and yeasts and moulds on Yeast Glucose Chloramphenicol agar (YGC agar; Biokar Diagnostics, Barcelona, Spain) at 25 °C for 5 days. Staphylococci and *Staphylococcus aureus* were counted on Baird Parker (Biokar Diagnostics, Barcelona, Spain) supplemented with egg-yolk tellurite emulsion after incubation at 37 °C for 2 days. Detection of *Listeria* spp. was applied on Palcam Selective agar (Biokar Diagnostics, Barcelona, Spain) according to IDF Standard 143A (IDF, 1995).

2.5. Physicochemical analysis

The pH of the cheese samples was measured using a pH-meter (632 pH meter, Metrohm Herisau, Switzerland). The moisture content was measured by heating the samples at 105 °C to constant weight (ISO, 2920/IDF 058, 2004). Fat content was determined by using the Gerber van Gulik method (Ardo and Polychroniadou, 1999) and expressed as fat in dry matter (FDM). Salt (NaCl) content was determined according to the standard method delineated by the International Dairy Federation (IDF, 1972). Total nitrogen (TN) was determined by the Kjeldhal method (ISO, 2014) and expressed as protein content (Nitrogen content multiplied by 6.38). Cheese proteolysis was followed by determining the water-soluble nitrogen (WSN) and the 12% trichloroacetic soluble nitrogen (12% TCA-SN) as described by Nega and Moatsou (2012). The concentrations of (WSN) and (12% TCA-SN) were expressed as percentage of total nitrogen (TN).

2.6. Antioxidant activity

Cheese methanol extracts evaluated for their antioxidant activity using the 2,2-diphenyl-1-picrylhydrazyl ($DPPH^+$) free radical scavenging assay described by Hilario et al. (2010) with some modifications. The cheese sample (20 g) was first extracted twice with 150 mL hexane, for the elimination of lipids, using an ultrasound water bath at the frequency of 35 KHz for 30 min. After filtration, the cheese residue was received and placed under nitrogen stream for the removing of solvent. Ultrasound extraction was repeated twice to the cheese residue with 100 mL methanol, and the methanol phases were recovered with centrifugation at 10 000 x g for 10 min at 4 °C (Sigma 3–18 K Centrifuge, Sigma Centrifuges, Newtown, UK). The total methanol extracts were left one day at 4 °C. Subsequently, they were dried with anhydrous magnesium sulfate, and concentrated using rotary evaporator at 30 °C (Buchi R-205, Labortechnik AG, Switzerland) until the volume of 4 mL. The methanol concentrates were stored at −18 °C for later analysis. For the ($DPPH^+$) free radical scavenging assay, 3.9 mL of $DPPH^+$ methanol solution (0.004% w/v) was mixed with 0.1 mL of cheese methanol extract. The blank solution was prepared using 0.1 mL of methanol instead of cheese extract. The solutions' absorbance was recorded at 517 nm for time zero and after 60 min. The decrease in the absorbance of the solutions was expressed as Radical Scavenging Activity (RSA%) given from the following equation: $RSA\% = [(At_0\text{-}At_{end})/At_0] \times 100$, where At_0 is the start absorbance at time zero, and At_{end} is the final absorbance after 60 min. The RSA% values of the cheese extracts were compared to the ones of standard Trolox solutions (6-hydroxyl-2,5,7,8-tetramethylchroman-2-carboxylic acid), at the concentrations of 10, 50 and 200 mg/L, under the same experimental procedure. Three repetitions were performed for each sample.

2.7. Color measurement

To measure the cheese color changings during storage, reflected color was measured at 1, 10, 20 and 30 days. A Minolta CR-300 colorimeter (Minolta Co., Osaka, Japan) with a calibrated plate (Minolta 11333110) with Y = 93.1, x = 0.3160, and y = 0.3323 was used. Commission International d' Eclair age (CIE) standard illuminant D65 and an angle vision of 10° were used. CIE L^*, a^*, and b^* coordinates were obtained from the saffron milk extracts. L^* corresponds to brightness (0 = black, 100 = white), a^* value to the red-green component (-a = green, +a = red) and b^* value (-b = blue, +b = yellow) represents the yellow-blue component. Color was directly measured on the surface of a standard amount of soft cheese which filled a petri plate, using a white background. For each cheese sample, five measurements were obtained, at each sampling time.

2.8. Sensory analysis

Twelve expert panelists from the staff of Dairy Science Laboratory of Agricultural University of Athens evaluated the cheese samples on 1st, 10th and 20th day of cold storage for appearance, body and texture, and flavor, using a 10 - point scale, ranging from 0 (poor) to 10 (best), defining the minimal and the maximal values for the quality assessment of the attributes described. More importance was given to the flavour and body and texture than to appearance of the cheese, by multiplying their scores by 5 and 4, respectively as advised by ISO (2009). The total score was obtained by adding the scores of the three attributes. *Excellent* was characterized the cheese graded with a total score of 100.

2.9. Statistical analysis

Data analysis was performed with Statgraphics Centurion XVI.I software (1995, Manugistics, Inc., 133 Rockville, Maryland 20852, USA). Comparisons among means were performed by ANOVA followed by LSD test for the estimation of significant differences. Differences were considered significant for P values < 0.05.

3. Results and discussion

3.1. Microbiological analysis

Regarding the quality of the pasteurized ovine milk used in cheese making, the mean value of TBC was 4.27 log cfu/mL, while none of the other examined groups was detected. Considering the microbial quality of saffron solution used for the milk supplementation, the counts of TBC, enterococci, micrococci, yeasts/moulds and coliforms were 5.06, 1.77, 2.70, 3.25and 1.5 log cfu/mL respectively. Spices may be highly contaminated with moulds, yeasts and bacteria at high microbial load of 3–8 log cfu/g (Ordoudi and Tsimidou, 2004) but their usage in small quantity in food manufacture, eliminates the health and product risk. The evolution of the studied microbial groups in cheeses during the storage period of 30 days at 4 °C is presented in Fig. 1. The TBC group, in all cheese samples during cold storage, followed a similar pattern of growth. Specifically, in the first day after manufacture, TBC counts reaching 9 log units were not significantly different ($P > 0.05$) (Fig. 1). After 10 days of storage, TBC counts dropped in all cheeses and during the rest storage time an additional gradual reduction, was observed, inversely to the saffron concentration (Fig. 1). Especially, in the case of saffron cheese C, this group was found at 3.60 log counts lower, than that in the control cheese, which may be attributed to a negative correlation to the increasing levels of saffron. The lactococci (starter) counts in all cheeses were not significantly different the first 10 days of storage ($P > 0.05$) with a ca 9 log count, but during the remaining storage time, a significant drop of 1–2 log counts($P < 0.05$) was observed in saffron cheeses only (data not shown). According to Litopoulou-Tzanetaki and Tzanetakis (2011) the lactic acid bacteria (LAB) population in a similar soft PDO cheese named Katiki Domokou, was 7 log counts after 30 days of storage. Counts of enterococci were found at the same level, at ca 4 log counts on the first day of storage in all cheeses, but on the 10th day were detected at 3.80 log counts only in the control cheese (Fig. 1). In general, this group of bacteria is a major component of the bacterial population of Greek cheeses (Nikolaou et al., 2002) and their presence is related with hygienic practices during cheese manufacture (Andrighetto et al., 2001). Coliforms were also found at their peak on 24 h of production, being negatively correlated with the level of saffron concentration in all cheeses as shown in Fig. 1. After ten days of storage, this group was not detectable in all cheeses. Micrococci were detected at the first day only in saffron cheeses A, B and C at 1.00, 2.30 and 4.44 log cfu/g, respectively and thereafter were not detected as shown in Fig. 1. Their presence could be attributed to their initial presence in the saffron extract used for cheese fortification, of 2.7 log cfu/g as reported above. Members of the genus Micrococcus have been detected as endophytes on several plants (Prakash et al., 2014). *Staphylococcus aureus* and *Listeria* spp. were not detected at all cheeses during storage and this was in accordance with Taracki et al. (2005), who studied a similar cheese supplemented with spices. Given that the cheese physicochemical parameters like pH, moisture and salt in moisture were not different in all cheeses during storage, the results indicate that the saffron supplementation could be the factor of microbial group reduction. Nevertheless, lactococci estimated at high level of 7 log count at the end of storage. According to Ledenbach and Marshall (2009) and Büchl and Seiler, (2011), yeast and moulds are related with off-flavors and gassy appearance in fresh cheeses without aging, unlike in matured cheeses where yeasts are contributed positively to the cheese flavor and aroma. Their presence is also reported by Litopoulou-Tzanetaki and Tzanetakis (2011) when examining the Touloumotyri cheese which is manufactured by a similar technology. The intrinsic cheese parameters and the storage temperature may favor the growth of yeasts according to Wojtatowicz et al., (2001). In the frame of this study, although yeasts were not detected on the first day in all cheeses, surprisingly, on the 10th day of storage, they were counted over the 4 log cfu/g and continously increased during the whole storage period reaching the final counts of about 6 log cfu/g (Fig. 1). The initial absence of yeasts in the saffron and control cheeses and the simultaneous appearance and equal evolution of this group during storage indicates that saffron extract supplementation was not the main source of yeast contamination, despite their initial presence in it. According to the microbial groups evolution, it is obvious that the 20 days of cold storage at 4 °C is presented as a critical time with regards to the quality and the safety of the studied cheeses, mainly due to the high counts of yeasts and molds which grow on the surface and deteriorate the cheeses.

3.2. Physicochemical characteristics

The physicochemical characteristics and the proteolysis level of cheeses are shown in Table 1. No significant differences were found in all treatments ($P > 0.05$) in regards to moisture, total protein, salt and fat content of the cheese samples during storage. The addition of saffron extract solution in milk did not seem to affect those parameters after cheese manufacture and during its storage (Table 1).

The moisture content and the fat in dry matter content of all cheeses (control and saffron cheeses) were found to meet the Greek Standard (≤75% moisture and ≥40% FDM content) for fresh spreadable cheese (Greek Codex Alimentarius, 2003). Similar FDM values have also been mentioned for similar traditional fresh cheeses such as for *Anevato* cheese (Xanthopoulos et al., 2000) and for *Galotyri*-type cheeses (Katsiari et al., 2009). Concerning the salt in moisture content of cheese samples which ranged from 1.37 to 1.66%, no significant differences ($P > 0.05$) were found among the examined cheeses (Table 1).

Regarding pH values, saffron cheeses revealed a similar decrease in pH value ($P > 0.05$) on day 1 which remained stable during the whole

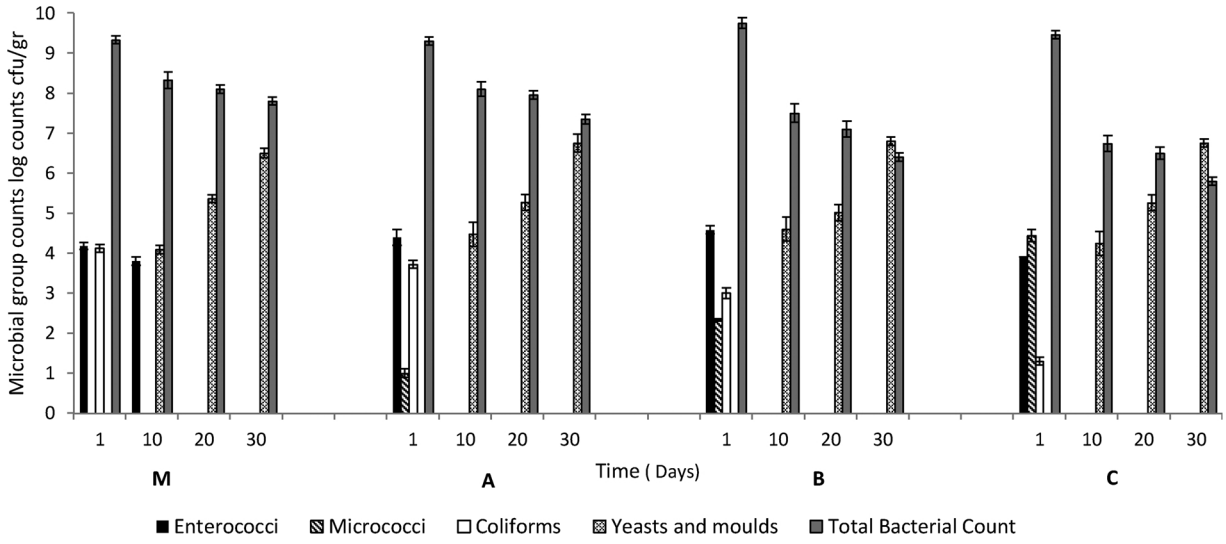

Fig. 1. xxx.

cold storage. The pH values of saffron cheeses ranged from 4.36 to 4.13 and showed that the starters' growth and their acidification activity was not affected by the saffron addition. Similar pH values have been reported by Xanthopoulos et al., (2000) in *Anevato* cheese, a fresh cheese made from raw ovine or goat milk without the addition of starter cultures. Moreover, in pressed cheeses, the saffron addition had no significant effect on pH values at any stage of cheese ripening (Licón et al., 2012).

Regarding the total protein content of cheeses, no significant differences were found. The protein content of the examined cheeses was found to be almost constant during their storage and ranged from 13.10 to 14.20% (Table 1). The water-soluble nitrogen (WSN) fraction of cheese is known to be used often for the measurement of cheese proteolysis (Christensen et al., 1991). Regarding nitrogen fractions of water soluble nitrogen (WSN) and the soluble nitrogen in 12% trichloro-acetic solution (12% TCA-SN), no differences were observed during storage up to 20 days. However, on the 30th day of cheese storage, a significant increase of the TCA-SN fraction was observed,

Table 1
Physicochemical characteristics of control and saffron cheeses (mean values ± standard error, n = 3).

		Storage time (days)			
Parameter	Cheese[1]	1	10	20	30
Moisture	Control	66.71 ± 0.55	67.47 ± 1.52	66.99 ± 0.47	68.47 ± 2.23
(% w/w)	A	66.69 ± 0.47	67.46 ± 1.12	67.74 ± 0.75	68.95 ± 2.27
	B	65.65 ± 1.21	66.18 ± 1.15	64.71 ± 2.27	68.43 ± 1.75
	C	66.54 ± 1.08	67.04 ± 1.45	65.34 ± 1.72	67.05 ± 1.12
FDM	Control	46.30 ± 0.67	48.89 ± 1.12	46.15 ± 0.85	46.09 ± 0.65
(% w/w)	A	47.89 ± 0.32	47.24 ± 0.21	44.26 ± 1.14	45.40 ± 0.53
	B	46.39 ± 0.14	45.88 ± 1.12	45.04 ± 1.35	45.83 ± 0.45
	C	47.18 ± 0.53	45.20 ± 1.14	44.13 ± 1.12	45.78 ± 0.34
Salt in Moisture	Control	1.47 ± 0.02	1.5 ± 0.08	1.48 ± 0,04	1.37 ± 0,12
(% w/w)	A	1.65 ± 0.05	1.66 ± 0.03	1.42 ± 0.14	1.44 ± 0.06
	B	1.45 ± 0.12	1.50 ± 0.05	1.58 ± 0.03	1.43 ± 0.13
	C	1.58 ± 0.06	1.54 ± 0.03	1.61 ± 0.06	1.52 ± 0.11
Protein	Control	12.10 ± 0.52	12.91 ± 0.76	13.71 ± 1.17	13.58 ± 1.06
(% w/w)	A	13.49 ± 1.14	14.67 ± 1.34	14.54 ± 1.10	14.32 ± 1.12
	B	13.10 ± 0.78	14.15 ± 1.23	14.65 ± 1.45	14.50 ± 1.15
	C	13.20 ± 1.04	13.95 ± 0.75	14.25 ± 1.05	14.20 ± 0.95
WSN/TN	Control	9.85 ± 0.45	9.80 ± 0.34	9.90 ± 0.75	10.00 ± 0.34^{x}
(% w/w)	A	8.80 ± 0.65^{a}	8.40 ± 0.85^{a}	8.60 ± 0.83^{a}	10.00 ± 0.43^{b,x}
	B	8.40 ± 0.75^{a}	8.83 ± 0.45^{a}	8.76 ± 0.26^{a}	10.85 ± 0.24^{b,x}
	C	8.80 ± 0.54^{a}	8.10 ± 0.64^{a}	8.60 ± 084^{a}	11.42 ± 0.32^{b,y}
12% TCA-SN/TN	Control	4.80 ± 0.16	4.90 ± 0.25	4.40 ± 0.54	5.10 ± 0.74^{x}
(% w/w)	A	3.60 ± 1.12^{a}	3.50 ± 1.23^{a}	4.60 ± 0.25^{a}	8.50 ± 1.23^{b,y}
	B	3.70 ± 1.42^{a}	3.53 ± 1.45^{a}	4.57 ± 0.72^{a}	9.86 ± 0.85^{b,z}
	C	3.60 ± 1.48^{a}	3.50 ± 1.23^{a}	4.60 ± 0.54^{a}	10.56 ± 0.54^{b,z}
pH	Control	4.31 ± 0.02	4.27 ± 0.11	4.25 ± 0.05	4.31 ± 0.06
	A	4.36 ± 0.05	4.13 ± 0.25	4.20 ± 0.12	4.27 ± 0.07
	B	4.35 ± 0.08	4.20 ± 0.12	4.24 ± 0.14	4.27 ± 0.08
	C	4.32 ± 0.01	4.22 ± 0.14	4.22 ± 0.011	4.34 ± 0.05

$^{a\ b\ c}$Means (± SE, n = 3) within a row with different superscripts differ significantly ($P < 0.05$).
$^{x\ y\ z}$Means (± SE, n = 3) within a column with different superscripts differ significantly ($P < 0.05$).
FDM = Fat in dry matter; WSN/TN = water-soluble nitrogen/total nitrogen; 12% TCA-SN/TN = soluble nitrogen fraction at 12% trichloroacetic acid (TCA)/total nitrogen.
[1] M = Control ovine cheese without saffron; A, B and C = cheeses supplemented with saffron equivalent to 50, 75 and 100 mg/g respectively.

Table 2
DPPH Radical Scavenging Activity (%) of methanolic cheese extracts with different concentrations of saffron and standard TROLOX solutions (Values are means ± SE for n = 3).

Sample Cheese Extract	Concentration (mg/g) Saffron	RSA (%)
M	0	$14.33^{a} \pm 2.51$
A	50	$25.97^{b} \pm 4.47$
B	75	$27.74^{b} \pm 4.94$
C	100	$23.84^{b} \pm 4.29$
Standard Solution	Trolox	$1.02^{c} \pm 0.43$
	10	
	50	$5.12^{d} \pm 1.54$
	75	$23.32^{b} \pm 4.29$
	200	$70.23^{e} \pm 3.60$

Trolox = 6-hydroxyl-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid; $DPPH^{+}$ = 1, 1-diphenyl-2-picrylhydrazyl; RSA = Radical Scavenging Activity.
[a-d]Means within a column with different superscripts differ significantly ($P < 0.05$).

positively correlated with the saffron concentration, in contrast with the control cheese, where the 12% TCA-SN soluble nitrogen fraction remained constant. This may be attributed to the peptidase activity of saffron which is known as a cysteine protease in *Crocus sativus* (Iqbal et al., 2012). Cysteine protease of *Crocus sativus* (CPCS) belongs to the family of papain cysteine peptidases with a wide action field either as a broad-spectrum protease (papain-like) or as an endopeptidase, di-peptidase and as an endo and exo-peptidase. The structure of the CSCP has bonds with the cysteine papain of plant or animal origin and the enzyme is active in a wide range of pH and temperature. Similar results have been reported by El-Aziz et al., (2012) in ginger extract-fortified soft cheese where a significant increase in the WSN/TN and tyrosine contents was observed after 6 weeks of storage. In a pressed ovine saffron cheese studied by Licón et al. (2012), slight differences in proteolysis rate were observed although they were not evident by the end of the cheese ripening period.

3.3. Antioxidant activity

Table 2 shows the (%) Radical Scavenging Activity (RSA%) of the cheese methanol extracts in relation to saffron concentration. The RSA% values are also presented for standard Trolox solutions at different concentrations. Significant differences ($P < 0.05$) on antioxidant capacity between the control cheese extract and the saffron-cheese extracts were observed. On the other hand, no significant differences were found between the cheeses with different saffron concentrations. The weakest radical scavenging activity of 14.33% was exhibited by the control cheese M without saffron, whereas the strongest activity (27.74%) was exhibited by the methanolic extract of cheese B at a saffron concentration equivalent to 75 mg/L. The RSA% for the Trolox solutions at concentrations between 10–200 mg/L ranged from 1.02 to 70.23%. According to the results, the methanolic extract of saffron cheese A (50 mg/L) displayed better antioxidant capacity than of the Trolox solution at equivalent concentration as shown in Table 2. In higher concentrations, Trolox showed better ability to quench the DPPH radical than saffron cheese extracts. The antioxidant capacity of saffron cheeses was not significantly increased ($P > 0.05$) with the increase of saffron concentration in milk making. This was in accordance with observations obtained in solutions of pure saffron colorings (apocarotenoids) in which the antioxidant activity increased until a certain point and then started to decrease at higher colorings' concentration (Kanakis et al., 2007). Moreover, the saffron cheese A (50 mg/L) was evaluated for its antioxidant activity during storage as it was the cheese with the best rating in sensory evaluation. Results showed that despite its higher initial RSA% value of 25.97, a gradual decrease was observed during its 30-day storage reaching 12.58, which was not statistically different ($P > 0.05$) from the corresponding value 14.33 of control cheese M. These results were in accordance with those obtained by El-Din et al., (2012) in low fat UF-soft cheese samples supplemented with thyme extract, where a higher antioxidant activity was observed gradually decreasing during its 30-day storage but finally remained higher than control samples. Similarly, according to Lee et al., (2016) the antioxidant activity of Cheddar-type cheese fortified with *Inula Britanica* extract decreased with increasing storage period correlated with a decrease in total phenolic content. Unlike the saffron cheese A, the control fresh cheese M seems to keep its antioxidant capacity during storage displaying RSA% values ranged from14.33 to 19.01 without being statistically different ($P > 0.05$) (data not shown).

Table 3
Mean values $L^{*}a^{*}b^{*}$ coordinates from control and saffron cheeses according to the International Commission on Illumination (CIE).

Cheese[1]	Storage (Days)	Color coordinate a^{*}	b^{*}	L^{*}
Control	0	-2.706^{f}	6.997^{b}	90.658^{abc}
	10	-0.687^{g}	2.821^{a}	96.944^{d}
	20	-3.416^{de}	2.850^{a}	89.354^{abc}
	30	-4.349^{d}	12.607^{b}	89.204^{abc}
A	0	-7.882^{bc}	29.754^{c}	89.398^{abc}
	10	-7.029^{c}	27.520^{c}	94.382^{bc}
	20	-7.920^{bc}	30.256^{cd}	89.137^{abc}
	30	-8.412^{abc}	31.350^{cde}	86.323^{abc}
B	0	-8.571^{cde}	35.310^{def}	88.857^{cd}
	10	-8.249^{abc}	33.908^{ef}	91.849^{abc}
	20	-9.121^{ab}	37.213^{fg}	85.981^{ab}
	30	-9.042^{abc}	36.717^{fg}	86.348^{abc}
C	0	-8.896^{abc}	39.129^{fg}	88.280^{abc}
	10	-8.805^{abc}	39.045^{fg}	91.576^{cd}
	20	-9.381^{a}	41.173^{g}	85.170^{a}
	30	-9.321^{ab}	41.107^{g}	85.442^{a}

L^{*}: luminance (ranges from 0 for black to 100 for white); a^{*} = a color's position between red and green; b^{*} = a color's position between yellow and blue.
[a-g]Means within a column with different superscripts significantly differ ($P < 0.05$). The standard error for color coordinates was 0.50, 1.77 and 1.93 for a^{*}, b^{*} and L^{*} respectively.
[1] M = Control ovine cheese; A, B and C = ovine cheeses supplemented with saffron equivalent to 50, 75 and 100 mg/g respectively.

3.4. Color

In Table 3 the mean values and standard deviation for CIE L^{*}, a^{*}, b^{*} coordinates are shown. Regarding the L^{*} values which indicate lightness, no significant affection was shown ($P > 0.05$), neither with the storage period of 30 days, nor with the saffron concentration. The latter was opposed to the findings of Licón et al. (2012) concerning pressed ovine cheeses, where the L^{*} coordinate values were decreased. Changes in a* and b* coordinates between the control and saffron cheeses were apparent as saffron concentration was increased. Regarding the a^{*} coordinate which indicates the red/green color, the control values ranging from (-2.7) to 4.34 were significantly higher than those of saffron-cheeses ranging from (-7.02) to (-9.32) due to the red pigmentation of saffron. This was in accordance with Noh et al., (2013) study concerning food supplementation with spices. Nevertheless, no significant difference ($P > 0.05$) was revealed in each saffron cheese during storage time. On the contrary, the coordinate b^{*}, which indicates the yellow/blue color, was the parameter that increased with the saffron concentration, suggesting that cheese color gets yellower when saffron increases. Nevertheless, statistical significant difference ($P < 0.05$) for this color parameter was observed between A and C cheeses, but not between B and C cheeses. This indicates that the lower saffron concentration of 50 mg/L gives a less yellowish color degree to cheese A and an almost similar yellow hue in cheeses B and C as it is shown in

Table 4
Sensory analysis of saffron cheeses (mean values ± standard error).

Cheeses[1]	Storage period (days) 1	10	20
	Appearance (10)*		
M	9.18 ± 0.17[a]	9.25 ± 0.17[a]	9.18 ± 0.17[a]
A	8.92 ± 0.21[a]	9.15 ± 0.36[a]	9.10 ± 0.21[a]
B	8.35 ± 0.18[b]	8.45 ± 0.18[b]	8.20 ± 0.18[b]
C	7.54 ± 0.33[c]	7.78 ± 0.33[c]	7.85 ± 0.33[c]
	Flavor (50)*		
M	41.84 ± 0.16[a]	40.65 ± 0.16[a]	40.21 ± 0.24[a]
A	40.93 ± 0.18[a]	39.34 ± 0.25[a]	41.36 ± 0.27[a]
B	38.46 ± 0.24[b]	37.53 ± 0.18[b]	36.43 ± 0.18[b]
C	31.57 ± 0.26[c]	30.85 ± 0.23c	30.52 ± 0.16[c]
	Body & Texture (40)*		
M	32.42 ± 0.26	31.85 ± 0.34	32.4 ± 0.24
A	31.65 ± 0.28	31.64 ± 0.28	31.6 ± 0.18
B	30.54 ± 0.35	30.53 ± 0.31	30.5 ± 0.23
C	30.82 ± 0.43	29.86 ± 0.35	30.8 ± 0.17
	Total Score (100)*		
M	83.38 ± 0.71[a]	81.75 ± 0.67[a]	81.79 ± 0.65[a]
A	81.42 ± 0.67[a]	80.13 ± 0.89[a]	82.06 ± 0.66[a]
B	77.25 ± 0.77[b]	76.22 ± 0.67[b]	75.13 ± 0.59[b]
C	68.84 ± 1.02[c]	67.85 ± 0.91[c]	69.17 ± 0.66[c]

[a-c]Means in the same column with the same letter were not significantly different ($P > 0.05$).

[1] M = Control ovine cheese; A, B and C = cheeses supplemented with saffron equivalent to 50, 75 and 100 mg/g respectively.

* Evaluated using a 10-point scale, ranging from 0 (poor) to 10 (best), where flavour and body and texture scores multiplied by 5 and 4, respectively.

Table 3. However, no change of this coordinate was observed in all samples during storage. Similar results had been reported by Licón et al. (2012) for the pressed ewe milk cheese with saffron, where its yellowish color was increased with the saffron concentration. The above color results of saffron cheeses indicate the stability of crocetin esters from oxidation during storage.

3.5. Sensory evaluation

The results of sensory evaluation of control and saffron cheeses during storage period at 4 °C are presented in Table 4. In detail, the panelists evaluated the control cheese equally with the saffron cheese A (50 mg/L) for all tested attributes and better than the saffron cheeses B and C. Saffron cheese C with the higher saffron concentration, received the lowest scoring ($P < 0.05$), for all sensory characteristics except texture. There were no clear differences between control and saffron cheese samples in texture scores, which agreed with the similar physicochemical characteristics during the storage. After the 20th day of storage, a gradual decrease in all sensory characteristics was noticed for all cheeses (data not shown), which should be attributed to the yeast and moulds' significant increase, as was shown in Fig. 1.

4. Conclusion

Fresh ovine cheese produced with saffron supplementation characterized by the enhanced growth of *Lactococcus lactis* strains used as starter culture, which was the predominant microbial group. The total and lactococci bacteria counts were not differentiated in all cheeses, immediately after manufacturing, however a significant decrease of populations was observed in saffron cheeses during storage. In addition, the saffron cheese exhibited a more intensive antimicrobial activity against coliforms and enterococci. Saffron cheeses didn't show any changes in cheese composition. An increasing proteolysis rate and enhanced antioxidant activity, progressively reducing during storage. The sensory analysis showed that the less saffron fortification of 50 mg/L, resulted to an acceptable fresh saffron cheese, which kept its traditional taste and aroma. Based on the above, saffron spice could be successfully used as a natural colorant in fresh cheese manufacture providing also antimicrobial and functional properties.

Conflict of interests

None.

Acknowledgements

Authors would like to thank the Laboratory of Dairy Research technicians, H. Grammatikas and Th. Paschos for their contribution to the cheese making experiments.

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