Chemical, microbiological, textural, color, and sensory characteristics of pressed ewe milk cheeses with saffron (*Crocus sativus* L.) during ripening

C. C. Licón,* M. Carmona,† A. Molina,* and M. I. Berruga*¹

*Departamento de Ciencia y Tecnología Agroforestal y Genética, ETSIA-IDR, Universidad de Castilla-La Mancha, Campus Universitario 02071, Albacete, Spain

†Albacete Science and Technology Park, Campus Universitario 02071, Albacete, Spain

ABSTRACT

Adding saffron to dairy products represents an innovative practice to introduce them to niche markets. This paper represents a contribution to this field, as few studies have evaluated the influence of this spice on general aspects and ripening parameters of cheese. In this work, pasteurized ewe milk pressed cheeses with saffron were made to study compositional, microbiological, color, textural, and sensory characteristics in relation to saffron concentration and ripening time. The main changes were observed on sensory characteristics and color. In addition, compositional, textural, and microbiological changes could be observed; among them, saffron cheeses were firmer and more elastic but less prone to fracture. A remarkable result that could lead to further studies is that saffron addition slightly slowed down growth of total and lactic acid bacteria. This resulted in a slightly lower rate of pH decrease during pressing and, as a consequence, lower salt and water content. Compositional differences were not evident by the end of the ripening period.

Key words: ewe milk cheese, saffron color, cheese ripening, texture

INTRODUCTION

Sheep milk production is of high importance in Mediterranean countries, especially Greece, Italy, and Spain, where cheese is one of the main food products in the diet. In Spain, 99% of ewe milk production is converted into cheese, either pure or mixed with other types of milk. In 2010, 44,800 tons of pure ewe milk cheeses were produced, a figure which represents only 14.83% of the total cheese production in Spain (FENIL, 2011). Ewe milk cheese is mostly produced on a small local scale, so most of these cheeses are not as competitive as cow milk cheeses. This small industry must coexist with bigger

ones and introduction into niche markets is mandatory to remain competitive (Dubeuf et al., 2010). Innovation in cheese has been in part driven by addition of several ingredients, such as colorants, spices, leaves, flavor, and aroma agents. A common practice consists in adding a natural yellow to orange color to some cheeses using spices such as annatto, which is used in Cheddar and Mimolette manufacturing, among others (Harbutt, 2010). The extraction of annatto has been widely studied (Preston and Rickard, 1980; Chisté et al., 2011) in terms of application to different matrices (Berset and Marty, 1986; Calvo and Salvador, 2000), color stability (Shumaker and Wendorff, 1998; Martley and Michel, 2001), and degradation (de O. Rios et al., 2005).

Although in other cheeses different spices are used to obtain a yellow to orange color similar to paprika and saffron, studies concerning the coloring influence of these ingredients on different cheese attributes are scarce. In the particular case of Piacentinu Ennese, which is one of the most well-known saffron cheeses, there are studies regarding physicochemical differences between artisanal and industrial cheeses and between cheeses produced with saffron from different origins (Horne et al., 2005; Carpino et al., 2008). Nevertheless, no studies have evaluated the influence of saffron on the ripening process.

The first attempt to study saffron behavior in a dairy matrix has recently been published (Licón et al., 2012). This work evidenced the influence of milk composition, namely fat, on the final color of saffron milk extracts. Moreover, a simple and short extraction method was proposed, optimizing its addition to cheesemaking. This fact is important because saffron remains an expensive spice, mostly because it is harvested by hand. Besides, compounds responsible for saffron color are highly water-soluble carotenoids, known as crocetin esters, and with milk being an emulsion, a study regarding saffron behavior in the milk was mandatory.

Saffron not only gives color to food but also flavor and aroma, a rare combination for a single spice. Saffron's characteristic bitter taste is given by picrocrocin, whereas the aroma mainly comes from safranal (Car-

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¹Corresponding author: mariaisabel.berruga@uclm.es

mona et al., 2006). In addition, evidence has been presented by Licón et al. (2010) concerning the biomedical properties of saffron in humans that could help with medical problems such as Alzheimer's disease, depression, sexual behavior dysfunction, and infertility.

To diversify ewe milk cheeses and promote consumption of both traditional products, this study attempts to determine whether saffron addition alters the main compositional, microbiological, textural, and sensory characteristics in pure ewe milk cheese and its possible influence on the ripening process.

MATERIALS AND METHODS

Experimental Design

Four different batches of pressed ewe milk cheese were made. Each one consisted in making 4 vats of cheese (300 L) from the same milk tank. These 4 vats included the control vat and 3 vats with saffron added. Saffron addition to the first vat was labeled S, because its exact concentration is protected by a pending patent (Berruga Fernández et al., 2009). The second vat, referred to as 2 × S, had 2 times the S concentration, and the third vat contained 3 times the S concentration (3 × S).

Nineteen pieces (~3 kg each) were taken from each vat and ripened. Eight ripening stages were fixed at 3, 15, 30, 60, 90, 120, 150, and 180 d for analysis and 2 cheese pieces at each stage were used. The cheese pieces were divided for their analysis as follows: the microbiological sample was obtained from the internal part of the first piece of cheese and after that, cheeses were cut in halves. From the 4 halves, 2 halves were used for texture (one half from each piece), one half for composition, a quarter of a piece for pH, and the last quarter for color determination. In addition, on d 60, 120, and 180, an additional third piece was used for sensory analysis.

Cheesemaking

Cheeses were manufactured at a local factory (Que-sería Campo Rus, Cuenca, Spain), using Manchega breed ewe milk from their own supply. The milk composition (g/100 g) had an average DM content (mean ± SD) of 19.43 ± 0.54 , fat content of 7.66 ± 0.38 , and protein content of 6.19 ± 0.17 . The total bacterial count was $5.91 \log \text{cfu/mL}$. Each vat used 300 L of pasteurized (72°C, 20 s) milk. Saffron addition was done after milk pasteurization, according to the pending patent (Berruga Fernández et al., 2009). For cheese manufacturing, a starter culture containing *Lactococcus lactis* ssp. *lactis*, *Lactococcus lactis* ssp. *cremoris*,

Lactococcus lactis ssp. *lactis* biovar diacetylactis, and *Streptococcus thermophilus* was added (CHOOZIT MA4001; Danisco, Sassenage, France) at 5 Danisco culture units (DCU)/100 L. Milk was held at 30°C during 20 min, adding 0.025% (vol/vol) of CaCl_2 and 0.01% (vol/vol) of lysozyme. Commercial rennet was used for coagulation (chymosin:pepsin, 94:6) at 0.023% (vol/vol). Thirty minutes later the curd was cut into 8- to 10-mm cubes and heated (37°C) and stirred for 45 min before whey separation. Curd was molded and pressed using a pneumatic press (100 kPa) for the amount of time needed for the pH to reach 5.2, which was between 4 and 5 h. Pieces of cheese weighing approximately 3 kg were obtained. Cheeses were placed in brine (18% NaCl wt/vol) for 18 h at 9°C. After that, they were kept in a cold chamber (9°C) for 48 h and then were ripened in a maturation chamber at $11 \pm 1^\circ\text{C}$ and relative humidity 85% for 180 d. Surface molds were removed when necessary.

Physicochemical Profile

Two centimeters of rind were removed from the cheeses prior to performing analyses. After that, cheese was grated to a uniform grain size (Moulinex, Lyon, France). A pH meter Crison GPL 22 (Crison, Barcelona, Spain) was used for pH determination using a Crison 5232 probe. Dry matter content was determined by drying the sample to a constant weight at 102°C following the International Dairy Federation standard (IDF, 1982). Fat content was determined by the Gerber-Van Gulik method according to the International Organization for Standardization (ISO, 2008). Total nitrogen was measured using the Kjeldahl method (AOAC, 1998) and protein was obtained multiplying by a factor of 6.38. Sodium chloride content was determined by using an infrared analyzer FoodScan (Foss Electric A/S, Hillerød, Denmark). Cheese nitrogen fractions were obtained according to IDF (1991). Water-soluble nitrogen (WSN), soluble nitrogen at pH 4.6 (SNpH4.6), soluble nitrogen in 12% TCA, and soluble nitrogen in 5% phosphotungstic acid (PTA) were obtained. Soluble nitrogen for these fractions was determined by the Kjeldahl method (AOAC, 1998). All determinations were done in duplicate.

Microbiology

To perform microbial analyses, samples were obtained from the internal part of the cheese with a cheese sampling tool. Two centimeters of rind was removed and cheese samples of 10 g were obtained. Samples were homogenized with 90 mL of sterile 0.1% (wt/vol) peptone water in a masticator (IUL SA, Barcelona, Spain)

for 60 s. Decimal dilution of the homogenates was prepared with 0.1% (wt/vol) peptone water (Scharlau, Barcelona, Spain) and seeded in the corresponding medium in duplicate using a Eddy Jet spiral plater (IUL SA). Total aerobic bacterial counts were performed on plate count agar (PCA; Panreac Química S.L.U., Barcelona, Spain) after incubation at 32°C for 48 h. Lactic acid bacteria were plated on M17 agar (Biokar Diagnostics, Barcelona, Spain) with incubation at 37°C for 48 h. Violet red bile agar with glucose was used for enterobacteria incubation (VRBG; Biokar Diagnostics) at 37°C for 24 h. Molds and yeasts were seeded in rose bengal agar (RB; Scharlau) and incubated at 25°C for 120 h. *Pseudomonas* was grown in cetrinide agar (base) with a cephaloridine Fucidin cetrinide (CFC) selective supplement (Biokar Diagnostics) incubated at 25°C for 120 h. Plates ranging from 30 to 300 cfu were selected for counting. A Counterstat Flash (IUL SA) was used for this purpose. Counts were expressed as common logarithm of colony-forming units per gram of sample.

Color Measurement

Tristimulus colorimetry [Commission Internationale d'Éclairage (CIE) $L^*a^*b^*$ color space, where L^* represents luminance and ranges from 0 for black to 100 for white, a^* represents the color's position between red/magenta and green, and b^* represents the color's position between yellow and blue], was used to measure cheese color. A Minolta Colorimeter CR-400 (Minolta, Osaka, Japan) with an illuminant D65 and a 10° observer was used. Calibration was done using a Minolta reference white plate 11333110 with $Y = 93.1$, $x = 0.3160$, and $y = 0.3323$, where Y = luminance and x and y represent the color chromatic color coordinates in the chromaticity diagram of CIE 1931. Color was directly measured in the transversal surface of the cheese, immediately after cutting the cheese in halves. Three measurements were obtained for each piece of cheese. After that, a cheese section was exposed to air at ambient temperature (~21°C). Color was determined at 30 and 60 min after cutting.

Rheological Determinations

Uniaxial Compression. Before analysis, 2 cm of rind were removed. Cubes of $25 \times 25 \times 25$ mm were obtained using a cheese blocker (Boska Holland BV, Bodegraven, the Netherlands) from each cheese half. Cubes were kept at room temperature (~21°C) for 30 min before analysis. Uniaxial compression was carried out according to Pavia et al. (1999). Cubes previously lubricated with glycerin to avoid friction effects were compressed to 80% of their original height at a constant

temperature using a TA-TX2 texture analyzer (Stable Micro Systems Ltd., Godalming, UK) with a 245 N load cell and a crosshead speed of 1.3 mm/s. The analysis was carried out in 6 cubes for each half portion. True stress and true strain values were calculated according to Calzada and Peleg (1978).

Stress Relaxation. Samples were obtained in the same way as for uniaxial compression test (12 cubes lubricated with glycerin). The test was carried at a crosshead speed of 3.3 mm/s with a 245 N load cell to a 10% compression for 2 min (TA-TX2 texture analyzer; Stable Micro Systems Ltd.). The stress-relaxation curves were fit to a linear way based on a Maxwell model and rearranged according to Peleg (1979). From the equations obtained, e and r values were calculated (Juan et al., 2007). The e -values represent the equilibrium residual values of normalized relaxation stress when time $\rightarrow \infty$. The term r is related to an elastic behavior for any material.

Sensory Analysis

Sensory evaluation, regarding color and flavor, consisted of triangular and ranking tests carried out on different days. Tests were done at 60, 120, and 180 d of ripening. To avoid color influence on flavor, color and flavor tests were done separately in both tests (ISO, 2004). Thirty-four untrained panelists between 20 and 60 yr old from the University of Castilla-La Mancha (Albacete, Spain) were selected for the study. All panelists met the following criteria: cheese consumers, healthy, and not subject to food allergies. Sixteen out of the total were males and 18 were females. Tasting sessions were conducted in an illuminated, odor-free, and aerated room at room temperature. Cheese samples were sliced in triangles (~0.5-cm width), presented on white plates and coded with a random 3-digit number. The order in which the samples were given to the panelists in all sessions was randomized. Flavor tests were carried out using a red light to avoid color effect on flavor perception. Panelists were instructed to rinse their mouth with water and eat apple pieces between sampling. Session times were approximately 20 min each.

Triangular test was intended to determine if consumers were able to discriminate between control and saffron cheeses. Control and cheeses with the lowest saffron concentration (S) were used. Panelists were asked to try 3 cheese samples and identify which sample was different.

The aim of the ranking test was to establish if consumers were able to classify cheeses from the lowest to the highest saffron concentration, regarding color and flavor. For this purpose the 3 saffron cheeses were used.

Statistical Analysis

An ANOVA was performed using the SPSS version 17.0 statistical package (SPSS Inc., Chicago, IL) to determine the effects of saffron concentration and ripening time on each parameter studied ($P < 0.05$). The Tukey test at a significance level of $P < 0.05$ was used to determine differences between means for concentrations and ripening days. In addition, a general linear model (GLM) was performed to determine the effects of saffron concentration, ripening time and air exposure and the interactions between these parameters on L^* , a^* , and b^* coordinates. Triangular test results interpretation was based on the ISO (2004) method. Ranking test was analyzed according to Friedman test based on the χ^2 distribution. Differences between saffron concentrations were established using a least significant difference formula based on the t -test (Lawless and Heymann, 2010).

RESULTS

Chemical Analysis

Tables 1 and 2 show the physicochemical parameters and nitrogen fractions of the studied cheeses (control and saffron cheeses) at different ripening stages. The pH of the cheeses after salting was around 5.2. No significant differences were found in pH values at any stage of ripening due to saffron addition, even though control cheeses had slightly lower pH values. During ripening, changes in pH values were not significant with the exception of $2 \times S$ cheeses.

Regarding compositional profile, control cheeses showed lower ($P \leq 0.05$) DM values at d 3 and lower protein values at d 60 and 150. No significant differences were found in fat content due to saffron addition (Table 1) but lower salt content was observed ($P \leq 0.05$). The evolution of these parameters during ripening was similar for all types of cheeses. Dry matter increased ($P \leq 0.01$) during ripening; as a consequence of this, water loss and fat and protein content slightly increased with ripening time and salt content significantly increased ($P \leq 0.01$).

Regarding nitrogen fractions (Table 2), differences between control and saffron cheeses were not constant along ripening; however, at d 60, differences between samples were more evident. In general, control cheeses showed higher values for WSN, SNpH4.6, and 12% TCA fractions. The phosphotungstic acid fraction did not differ significantly between cheeses. These differences were less evident by the end of ripening period. All nitrogen fractions increased constantly ($P \leq 0.05$) during ripening.

Microbiology

No significant differences were found between control and saffron cheeses for enterobacteria, pseudomonae, yeasts, and molds (data not shown); however, total and lactic acid bacteria counts of control cheeses were slightly higher (Figure 1). These differences were only significant for specific days ($P \leq 0.01$). All microbiological groups studied dropped their counts during the ripening period ($P \leq 0.01$; data not shown). After manufacturing, the average total counts were between 8.5 and 9.3 log cfu/g and decreased to 7.0 log cfu/g at 180 d of ripening. Lactic acid bacteria were the predominant group, as expected, because cheeses were made from pasteurized milk. The counts ranged from 9.5 log cfu/g at 3 d to 7.2 log cfu/g after 180 d. A decrease in enterobacteria counts during ripening was observed. At d 3, cheeses had average counts of 2.3 log cfu/g but after 90 d they were absent (data not shown). Moreover, pseudomonads were absent starting from 15 d in all cheeses (data not shown). Yeasts and molds also decreased during ripening but their decreases were not as marked as the rest of the microorganisms; counts started at 2.0 log cfu/g and decreased to 0.7 log cfu/g (data not shown).

Color

Mean values and standard deviation obtained for CIE $L^*a^*b^*$ coordinates are shown in Table 3. In general, changes in L^* , a^* , and b^* coordinates were observed as saffron concentration was increased. The L^* coordinate values decreased with saffron addition after d 3 ($P \leq 0.05$); thus, saffron cheeses were less bright than control ones. This coordinate also decreased with ripening time ($P \leq 0.05$). This drop was more marked for saffron cheeses than for control ones.

Values of coordinate a^* were negative for all cheeses. A remarkable result is that control cheeses showed similar a^* values as $3 \times S$ cheeses. In addition, as saffron concentration was decreased, a^* values became more negative ($P \leq 0.001$), thus less red. Although changes during ripening showed significant differences ($P \leq 0.05$), except for $2 \times S$ cheeses, these changes were not very marked.

Coordinate b^* was the most influenced by saffron addition. Values increased with saffron concentration; as a result, saffron cheeses were more yellow ($P \leq 0.001$) than control cheeses. This increment was very marked between concentrations: control b^* values were around 14, whereas saffron cheeses values ranged between 33 and 51 (Table 3). Control cheeses did not show differences during the ripening period, whereas saffron

Table 1. Mean values $\pm$ standard deviation for pH and chemical composition from control and saffron cheeses

Parameter	Cheese ¹	Ripening time (d)								<i>P</i> -value
		3	15	30	60	90	120	150	180	
pH	Control	5.12 $\pm$ 0.01	5.24 $\pm$ 0.28	5.18 $\pm$ 0.09	5.19 $\pm$ 0.08	5.23 $\pm$ 0.17	5.17 $\pm$ 0.18	5.24 $\pm$ 0.06	5.19 $\pm$ 0.15	NS
	S	5.24 $\pm$ 0.13	5.30 $\pm$ 0.12	5.33 $\pm$ 0.14	5.27 $\pm$ 0.14	5.28 $\pm$ 0.15	5.24 $\pm$ 0.14	5.19 $\pm$ 0.17	5.23 $\pm$ 0.16	NS
	2 $\times$ S	5.24 $\pm$ 0.08 ^{ab}	5.32 $\pm$ 0.08 ^b	5.32 $\pm$ 0.11 ^{ab}	5.25 $\pm$ 0.05 ^{ab}	5.26 $\pm$ 0.13 ^{ab}	5.21 $\pm$ 0.13 ^{ab}	5.18 $\pm$ 0.13 ^{ab}	5.16 $\pm$ 0.06 ^a	*
	3 $\times$ S	5.22 $\pm$ 0.14	5.30 $\pm$ 0.14	5.40 $\pm$ 0.17	5.24 $\pm$ 0.11	5.24 $\pm$ 0.16	5.23 $\pm$ 0.12	5.18 $\pm$ 0.11	5.24 $\pm$ 0.11	NS
	<i>P</i> -value ²	NS	NS	NS	NS	NS	NS	NS	NS	
DM (g/100 g)	Control	52.93 $\pm$ 0.47 ^{a,x}	57.57 $\pm$ 3.15 ^{ab}	59.72 $\pm$ 3.03 ^{abc}	61.79 $\pm$ 4.07 ^{bcd}	63.55 $\pm$ 2.41 ^{bcd}	65.47 $\pm$ 3.53 ^{cd}	64.83 $\pm$ 2.29 ^{bcd}	67.80 $\pm$ 3.53 ^d	**
	S	58.17 $\pm$ 2.55 ^{a,y}	61.39 $\pm$ 2.31 ^{abc}	60.45 $\pm$ 2.74 ^{ab}	63.14 $\pm$ 2.98 ^{bcd}	63.95 $\pm$ 2.63 ^{bcd}	65.84 $\pm$ 2.52 ^d	65.64 $\pm$ 2.48 ^{cd}	67.48 $\pm$ 2.84 ^d	***
	2 $\times$ S	57.86 $\pm$ 1.08 ^{a,y}	60.20 $\pm$ 3.15 ^{ab}	59.92 $\pm$ 2.69 ^{ab}	62.32 $\pm$ 2.27 ^{bc}	63.63 $\pm$ 2.66 ^{abc}	64.98 $\pm$ 3.09 ^{cd}	64.81 $\pm$ 1.41 ^{cd}	67.11 $\pm$ 2.51 ^d	***
	3 $\times$ S	57.55 $\pm$ 3.44 ^{a,y}	59.72 $\pm$ 1.21 ^{ab}	60.50 $\pm$ 3.24 ^{abc}	61.65 $\pm$ 2.02 ^{bcd}	62.68 $\pm$ 1.72 ^{bcd}	63.96 $\pm$ 2.65 ^{cde}	64.62 $\pm$ 2.10 ^{de}	66.70 $\pm$ 2.45 ^e	***
	<i>P</i> -value	*	NS	NS	NS	NS	NS	NS	NS	
Fat (g/100 g)	Control	33.53 $\pm$ 0.03	33.38 $\pm$ 3.95	35.50 $\pm$ 0.71	35.50 $\pm$ 4.34	35.25 $\pm$ 2.66	35.88 $\pm$ 2.81	35.13 $\pm$ 0.63	35.38 $\pm$ 1.38	NS
	S	32.72 $\pm$ 3.52	33.75 $\pm$ 2.38	33.81 $\pm$ 2.67	34.06 $\pm$ 2.69	34.31 $\pm$ 2.79	36.06 $\pm$ 3.03	34.82 $\pm$ 1.98	35.38 $\pm$ 2.25	NS
	2 $\times$ S	32.71 $\pm$ 2.36	32.88 $\pm$ 3.53	33.13 $\pm$ 2.50	33.69 $\pm$ 1.73	33.81 $\pm$ 2.50	34.00 $\pm$ 2.73	34.56 $\pm$ 0.62	34.25 $\pm$ 2.27	NS
	3 $\times$ S	33.42 $\pm$ 3.62	32.75 $\pm$ 3.30	33.69 $\pm$ 2.39	32.75 $\pm$ 1.44	34.06 $\pm$ 1.99	36.06 $\pm$ 2.16	34.38 $\pm$ 1.27	35.13 $\pm$ 2.36	NS
	<i>P</i> -value	NS	NS	NS	NS	NS	NS	NS	NS	
Protein (g/100 g)	Control	20.14 $\pm$ 0.72	21.11 $\pm$ 1.65	21.51 $\pm$ 0.09	20.72 $\pm$ 0.19 ^x	21.65 $\pm$ 1.67	22.93 $\pm$ 2.36	23.12 $\pm$ 0.79 ^x	22.98 $\pm$ 0.55	NS
	S	23.39 $\pm$ 1.16	22.67 $\pm$ 1.88	23.01 $\pm$ 0.51	23.25 $\pm$ 0.86 ^y	23.95 $\pm$ 2.03	24.04 $\pm$ 1.10	25.02 $\pm$ 1.04 ^y	24.50 $\pm$ 0.75	NS
	2 $\times$ S	23.01 $\pm$ 1.04	23.11 $\pm$ 1.17	22.86 $\pm$ 0.84	23.31 $\pm$ 0.70 ^y	23.79 $\pm$ 1.25	24.77 $\pm$ 0.84	24.64 $\pm$ 0.62 ^{xy}	24.74 $\pm$ 2.25	NS
	3 $\times$ S	21.94 $\pm$ 1.76	23.03 $\pm$ 2.08	23.15 $\pm$ 0.77	22.69 $\pm$ 0.49 ^y	23.55 $\pm$ 2.24	23.97 $\pm$ 0.38	23.58 $\pm$ 0.97 ^{xy}	25.06 $\pm$ 0.46	NS
	<i>P</i> -value	NS	NS	NS	**	NS	NS	*	NS	
Salt (g/100 g)	Control	1.37 $\pm$ 0.03 ^a	1.60 $\pm$ 0.11 ^{ab,y}	1.79 $\pm$ 0.02 ^{bc,y}	1.96 $\pm$ 0.03 ^{bc,y}	2.11 $\pm$ 0.15 ^{c,y}	2.07 $\pm$ 0.22 ^{c,y}	2.09 $\pm$ 0.18 ^{c,y}	2.13 $\pm$ 0.27 ^{c,y}	***
	S	1.01 $\pm$ 0.25 ^a	1.30 $\pm$ 0.11 ^{b,x}	1.61 $\pm$ 0.06 ^{c,y}	1.63 $\pm$ 0.17 ^{c,x}	1.80 $\pm$ 0.01 ^{c,x}	1.77 $\pm$ 0.09 ^{c,xy}	1.70 $\pm$ 0.03 ^{c,x}	1.72 $\pm$ 0.13 ^{c,x}	***
	2 $\times$ S	0.93 $\pm$ 0.26 ^a	1.37 $\pm$ 0.10 ^{b,x}	1.43 $\pm$ 0.15 ^{b,x}	1.62 $\pm$ 0.18 ^{b,x}	1.65 $\pm$ 0.12 ^{b,x}	1.55 $\pm$ 0.15 ^{b,x}	1.61 $\pm$ 0.14 ^{b,x}	1.63 $\pm$ 0.21 ^{b,x}	***
	3 $\times$ S	0.78 $\pm$ 0.46 ^a	1.38 $\pm$ 0.21 ^{b,xy}	1.63 $\pm$ 0.14 ^{b,y}	1.70 $\pm$ 0.22 ^{b,xy}	1.76 $\pm$ 0.01 ^{b,x}	1.54 $\pm$ 0.15 ^{b,x}	1.81 $\pm$ 0.13 ^{b,x}	1.70 $\pm$ 0.06 ^{b,x}	***
	<i>P</i> -value	NS	*	***	*	***	***	***	**	

^{a-e}Means within a row with different superscripts differ ($P < 0.05$).

^{x,y}Means within a column with different superscripts differ ($P < 0.05$).

¹S = a specific concentration of saffron added to the cheese (Berruga Fernández et al., 2009); 2 $\times$ S = twice the concentration of S; 3 $\times$ S = 3 times the concentration of S.

²Significance differences between saffron concentration are indicated as follows: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

Table 2. Mean values $\pm$ standard deviation for nitrogen fractions from control and saffron cheeses

Parameter ¹	Cheese ²	Ripening time (d)								<i>P</i> -value
		3	15	30	60	90	120	150	180	
WSN/TN (g/100 g)	Control	7.37 $\pm$ 0.23 ^{a,z}	7.95 $\pm$ 1.58 ^a	10.41 $\pm$ 0.08 ^{ab}	17.91 $\pm$ 0.04 ^{c,y}	15.85 $\pm$ 2.84 ^{bc}	18.11 $\pm$ 2.18 ^{c,y}	20.60 $\pm$ 3.21 ^c	19.87 $\pm$ 2.53 ^c	***
	S	5.42 $\pm$ 0.70 ^{a,x}	7.77 $\pm$ 0.86 ^{ab}	9.48 $\pm$ 0.64 ^{bc}	12.16 $\pm$ 2.00 ^{cd,x}	13.99 $\pm$ 2.54 ^{de}	14.64 $\pm$ 1.74 ^{de,x}	16.90 $\pm$ 2.67 ^{ef}	20.57 $\pm$ 0.42 ^f	***
	2 $\times$ S	6.59 $\pm$ 0.27 ^{a,y}	6.65 $\pm$ 1.28 ^a	9.24 $\pm$ 0.38 ^{ab}	10.61 $\pm$ 0.37 ^{bc,x}	13.15 $\pm$ 0.59 ^{cd}	14.73 $\pm$ 0.36 ^{d, x}	18.19 $\pm$ 1.01 ^e	18.74 $\pm$ 2.81 ^e	***
	3 $\times$ S	6.21 $\pm$ 0.04 ^{a,xy}	6.57 $\pm$ 1.41 ^a	9.39 $\pm$ 0.55 ^a	13.27 $\pm$ 1.06 ^{b,x}	15.41 $\pm$ 2.06 ^b	15.77 $\pm$ 0.35 ^{bc,xy}	19.90 $\pm$ 2.28 ^d	19.41 $\pm$ 2.55 ^{cd}	***
	<i>P</i> -value ³	**	NS	NS	***	NS	*	NS	NS	
SNpH4.6/TN (g/100 g)	Control	7.74 $\pm$ 0.18 ^{a,y}	8.87 $\pm$ 2.23 ^a	11.10 $\pm$ 0.25 ^{ab,y}	16.21 $\pm$ 0.15 ^{cd,y}	14.18 $\pm$ 1.12 ^{bc}	17.81 $\pm$ 1.82 ^{cde,y}	20.15 $\pm$ 1.28 ^{de}	21.26 $\pm$ 0.76 ^e	***
	S	5.23 $\pm$ 0.16 ^{a,x}	8.63 $\pm$ 2.36 ^{ab}	9.51 $\pm$ 0.93 ^{ab,x}	11.64 $\pm$ 0.73 ^{bc,xy}	12.66 $\pm$ 3.79 ^{bcd}	14.35 $\pm$ 2.22 ^{bcd,x}	16.50 $\pm$ 3.63 ^{cd}	18.03 $\pm$ 3.35 ^d	***
	2 $\times$ S	5.51 $\pm$ 1.46 ^{a,xy}	7.01 $\pm$ 1.53 ^{ab}	9.30 $\pm$ 0.33 ^{ab,x}	11.45 $\pm$ 2.76 ^{bc,x}	11.82 $\pm$ 1.08 ^{bc}	14.93 $\pm$ 0.16 ^{cd,xy}	19.68 $\pm$ 2.47 ^d	20.29 $\pm$ 4.83 ^d	***
	3 $\times$ S	5.47 $\pm$ 0.93 ^{a,xy}	9.32 $\pm$ 4.90 ^{ab}	9.48 $\pm$ 0.57 ^{ab,x}	13.19 $\pm$ 1.45 ^{bc,xy}	13.83 $\pm$ 3.45 ^{bcd}	16.19 $\pm$ 0.28 ^{cd,xy}	19.36 $\pm$ 3.05 ^{cd}	19.66 $\pm$ 3.07 ^d	***
	<i>P</i> -value	*	NS	*	*	NS	*	NS	NS	
12% TCA/TN (g/100 g)	Control	7.51 $\pm$ 0.13 ^{a,y}	7.06 $\pm$ 2.79 ^a	6.97 $\pm$ 0.02 ^a	13.77 $\pm$ 0.01 ^{bc,y}	10.66 $\pm$ 1.29 ^{ab}	12.53 $\pm$ 1.32 ^{bc}	14.40 $\pm$ 0.37 ^{bc}	15.64 $\pm$ 0.26 ^c	***
	S	6.07 $\pm$ 0.73 ^{ab,x}	5.62 $\pm$ 0.59 ^a	5.45 $\pm$ 1.07 ^a	9.14 $\pm$ 0.39 ^{abc,x}	9.47 $\pm$ 2.50 ^{abc}	9.11 $\pm$ 3.79 ^{abc}	11.31 $\pm$ 3.37 ^{bc}	13.52 $\pm$ 2.84 ^c	***
	2 $\times$ S	6.80 $\pm$ 0.32 ^{ab,xy}	5.02 $\pm$ 1.47 ^a	5.06 $\pm$ 0.57 ^a	8.96 $\pm$ 2.38 ^{bc,x}	8.54 $\pm$ 0.53 ^{bc}	9.91 $\pm$ 0.36 ^{bc}	11.14 $\pm$ 1.56 ^{cd}	13.42 $\pm$ 2.30 ^d	***
	3 $\times$ S	6.01 $\pm$ 0.51 ^{a,x}	4.80 $\pm$ 1.48 ^a	5.41 $\pm$ 0.79 ^a	10.11 $\pm$ 1.35 ^{b,xy}	10.09 $\pm$ 1.65 ^b	10.83 $\pm$ 0.92 ^b	13.12 $\pm$ 2.69 ^b	13.15 $\pm$ 2.33 ^b	***
	<i>P</i> -value	*	NS	NS	*	NS	NS	NS	NS	
PTA/TN (g/100 g)	Control	1.37 $\pm$ 0.03 ^a	1.50 $\pm$ 0.02 ^a	1.77 $\pm$ 0.06 ^a	2.94 $\pm$ 0.10 ^{ab,y}	3.07 $\pm$ 0.91 ^{ab}	3.50 $\pm$ 1.05 ^{ab}	4.25 $\pm$ 0.78 ^b	4.42 $\pm$ 0.19 ^b	**
	S	2.34 $\pm$ 1.48 ^a	1.73 $\pm$ 0.07 ^a	1.91 $\pm$ 0.32 ^a	2.10 $\pm$ 0.47 ^{a,x}	2.65 $\pm$ 0.45 ^a	3.35 $\pm$ 0.32 ^{ab}	3.12 $\pm$ 1.20 ^{ab}	4.98 $\pm$ 0.90 ^b	***
	2 $\times$ S	1.42 $\pm$ 0.56 ^a	1.80 $\pm$ 0.13 ^{ab}	1.93 $\pm$ 0.62 ^{abc}	1.99 $\pm$ 0.07 ^{abc,x}	2.30 $\pm$ 0.29 ^{abc}	3.00 $\pm$ 0.13 ^c	2.87 $\pm$ 0.74 ^{bc}	4.65 $\pm$ 0.26 ^d	***
	3 $\times$ S	1.74 $\pm$ 1.05 ^{ab}	1.59 $\pm$ 0.07 ^a	1.69 $\pm$ 0.31 ^{ab}	1.91 $\pm$ 0.08 ^{ab,x}	2.32 $\pm$ 0.32 ^{ab}	2.78 $\pm$ 0.36 ^b	2.59 $\pm$ 0.36 ^{ab}	4.48 $\pm$ 0.17 ^c	***
	<i>P</i> -value	NS	NS	NS	**	NS	NS	NS	NS	

^{a-f}Means within a row with different superscripts differ ($P < 0.05$).

^{x,y}Means within a column with different superscripts differ ($P < 0.05$).

¹WSN/TN = water-soluble nitrogen/total nitrogen; SNpH4.6 = soluble nitrogen at pH 4.6; 12% TCA = soluble nitrogen at 12% TCA; PTA = phosphotungstic acid-soluble nitrogen.

²S = a specific concentration of saffron added to the cheese (Berruga Fernández et al., 2009); 2 $\times$ S = twice the concentration of S; 3 $\times$ S = 3 times the concentration of S.

³Significance differences between saffron concentration are indicated as follows: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

Table 3. Mean values $\pm$ standard deviation for Commission Internationale d'Éclairage (CIE) L*a*b* coordinates from control and saffron cheeses¹

Color coordinate	Cheese ²	Ripening time (d)								P-value
		3	15	30	60	90	120	150	180	
L*	Control	86.73 $\pm$ 0.70 ^c	86.34 $\pm$ 2.39 ^{c,y}	84.68 $\pm$ 1.06 ^{bc,y}	83.96 $\pm$ 0.78 ^{bc,y}	82.24 $\pm$ 2.07 ^{ab,y}	81.39 $\pm$ 2.12 ^{ab,y}	79.99 $\pm$ 0.38 ^a	80.00 $\pm$ 0.80 ^{a,y}	***
	S	86.52 $\pm$ 0.85 ^e	82.03 $\pm$ 0.66 ^{d,x}	81.39 $\pm$ 1.20 ^{cd,x}	80.69 $\pm$ 1.22 ^{bcd,x}	79.64 $\pm$ 1.44 ^{abcd,x}	78.60 $\pm$ 2.07 ^{abc,xy}	77.39 $\pm$ 3.20 ^a	77.89 $\pm$ 2.16 ^{ab,xy}	***
	2 $\times$ S	85.21 $\pm$ 1.63 ^e	80.90 $\pm$ 0.98 ^{cd,x}	81.03 $\pm$ 1.28 ^{d,x}	79.56 $\pm$ 1.36 ^{cd,x}	78.41 $\pm$ 1.47 ^{abc,x}	77.71 $\pm$ 2.18 ^{ab,x}	77.07 $\pm$ 1.93 ^{ab}	76.89 $\pm$ 1.81 ^{a,x}	***
	3 $\times$ S	86.81 $\pm$ 1.01 ^e	80.85 $\pm$ 0.93 ^{d,x}	80.43 $\pm$ 0.83 ^{d,x}	79.96 $\pm$ 0.70 ^{cd,x}	79.03 $\pm$ 0.75 ^{bcd,x}	78.07 $\pm$ 1.30 ^{abc,x}	77.07 $\pm$ 2.23 ^{ab}	76.60 $\pm$ 1.54 ^{a,x}	***
	P-value ²	NS	***	***	***	***	*	NS	*	
a*	Control	-3.72 $\pm$ 0.02 ^{a,z}	-2.95 $\pm$ 0.29 ^{b,z}	-2.92 $\pm$ 0.26 ^{b,z}	-3.13 $\pm$ 0.34 ^{ab,z}	-3.17 $\pm$ 0.20 ^{ab,z}	-3.28 $\pm$ 0.24 ^{ab,z}	-3.31 $\pm$ 0.18 ^{ab,z}	-3.33 $\pm$ 0.29 ^{ab,z}	*
	S	-4.99 $\pm$ 0.15 ^{ab,x}	-5.29 $\pm$ 0.18 ^{a,w}	-4.97 $\pm$ 0.10 ^{ab,x}	-4.96 $\pm$ 0.18 ^{ab,x}	-4.89 $\pm$ 0.18 ^{b,x}	-4.97 $\pm$ 0.21 ^{ab,x}	-5.00 $\pm$ 0.28 ^{ab,x}	-4.85 $\pm$ 0.30 ^{b,x}	**
	2 $\times$ S	-4.41 $\pm$ 0.16 ^y	-4.57 $\pm$ 0.18 ^x	-4.00 $\pm$ 1.02 ^y	-4.05 $\pm$ 0.40 ^y	-4.14 $\pm$ 0.36 ^y	-4.14 $\pm$ 0.27 ^y	-4.12 $\pm$ 0.14 ^y	-3.89 $\pm$ 0.32 ^y	NS
	3 $\times$ S	-3.80 $\pm$ 0.22 ^{a,z}	-3.81 $\pm$ 0.13 ^{a,y}	-3.46 $\pm$ 0.22 ^{ab,yz}	-3.39 $\pm$ 0.25 ^{bc,z}	-3.27 $\pm$ 0.24 ^{bc,z}	-3.31 $\pm$ 0.37 ^{bc,z}	-3.06 $\pm$ 0.22 ^{c,z}	-3.09 $\pm$ 0.30 ^{bc,z}	***
	P-value	***	***	***	***	***	***	***	***	
b*	Control	14.45 $\pm$ 0.07 ^w	12.03 $\pm$ 0.71 ^w	12.14 $\pm$ 0.77 ^w	13.05 $\pm$ 1.23 ^w	12.84 $\pm$ 1.33 ^w	12.79 $\pm$ 0.97 ^w	13.19 $\pm$ 0.91 ^w	13.50 $\pm$ 1.49 ^w	NS
	S	33.20 $\pm$ 0.81 ^{a,x}	35.35 $\pm$ 1.25 ^{b,x}	35.40 $\pm$ 0.83 ^{b,x}	35.34 $\pm$ 0.70 ^{b,x}	35.14 $\pm$ 0.94 ^{b,x}	35.71 $\pm$ 0.92 ^{b,x}	34.60 $\pm$ 0.87 ^{b,x}	35.00 $\pm$ 1.20 ^{ab,x}	***
	2 $\times$ S	41.51 $\pm$ 2.28 ^{a,y}	46.14 $\pm$ 0.72 ^{b,y}	45.64 $\pm$ 1.13 ^{b,y}	47.21 $\pm$ 2.88 ^{b,y}	46.79 $\pm$ 1.86 ^{b,y}	46.58 $\pm$ 1.11 ^{b,y}	45.85 $\pm$ 1.25 ^{b,y}	44.82 $\pm$ 1.05 ^{b,y}	***
	3 $\times$ S	47.15 $\pm$ 1.30 ^{a,z}	52.80 $\pm$ 1.18 ^{c,z}	51.56 $\pm$ 0.70 ^{bc,z}	52.36 $\pm$ 0.79 ^{bc,z}	52.03 $\pm$ 1.03 ^{bc,z}	52.25 $\pm$ 0.81 ^{bc,z}	52.00 $\pm$ 0.59 ^{bc,z}	51.10 $\pm$ 1.09 ^{b,z}	***
	P-value	***	***	***	***	***	***	***	***	

^{a-e}Means within a row with different superscripts differ ($P < 0.05$).

^{w-z}Means within a column with different superscripts differ ($P < 0.05$).

¹L* = luminance (ranges from 0 for black to 100 for white); a* = a color's position between red/magenta and green; b* = a color's position between yellow and blue.

²S = a specific concentration of saffron added to the cheese (Berruga Fernández et al., 2009); 2 $\times$ S = twice the concentration of S; 3 $\times$ S = 3 times the concentration of S.

³Significance differences between saffron concentration are indicated as follows: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

Figure 1. Mean values of total bacteria (A) and lactic acid bacteria (B) counts in control cheese (□), specific concentration of saffron-added cheese (S; Berruga Fernández et al., 2009; ■), 2 $\times$ S (twice the concentration of S) cheese (●) and 3 $\times$ S (3 times the concentration of S) cheese (▲) as a function of ripening time. Significance levels for each ripening time are indicated as follows: * $P < 0.05$ and *** $P < 0.001$. Samples marked by different letters (a and b) differed significantly.

cheeses only showed significant differences ($P \leq 0.05$) in the first days.

Regarding the effect of air exposure on color, GLM was done to establish if saffron concentration, ripening time, and air exposure affected these characteristics. Results are shown in Table 4. The models obtained by the GLM for the CIE L*a*b* coordinates and the 3 factors studied were significant ($P < 0.001$), except for ripening on b*. Interactions between factors were not statistically significant for any coordinate (data not shown). The coefficient of determination for coordinate b* was adequate by being closer to 90%. However coordinate L* and a* presented lower coefficients of determination; consequently, they could not be as prop-

Table 4. General linear model for Commission Internationale d'Éclairage (CIE) L*a*b* coordinates in control and saffron cheeses in terms of saffron concentration, ripening time, and air exposure¹

Model ²	Color coordinate		
	L*	a*	b*
R ²	65.766	34.723	88.491
SEE ³	2.799	0.700	4.370
P-value	0.000	0.000	0.000
Constant	86.290	−5.033	19.747
Saffron concentration ⁴	−0.892***	0.424***	11.675***
Ripening time ⁵	−0.055***	0.002***	
Air exposure ⁶	−0.072***	−0.009***	0.078***

¹L* = luminance (ranges from 0 for black to 100 for white); a* = a color's position between red/magenta and green; b* = a color's position between yellow and blue.

²Significance levels for each factor: *** $P < 0.001$.

³Standard error of the estimation.

⁴Saffron concentration was between 0 and $3 \times S$, where $3 \times S = 3$ times the concentration of a specific concentration of saffron (S) added to the cheese (Berruga Fernández et al., 2009).

⁵Ripening time was between 3 and 180 d.

⁶Air exposure was between 0 and 60 min.

erly estimated as b*. Exposure time was statistically significant ($P < 0.001$) for all color coordinates, confirming the visually observed color changes in control and saffron cheeses, but its effect was not as marked as saffron concentration. Cheeses resulted less bright and red and more yellow as exposure time was increased.

Rheological Determinations

Uniaxial Compression. Fracture stress and fracture strain obtained from the stress curves of control and saffron cheeses are shown in Figure 2A and 2B. Saffron addition and ripening time were factors that influenced ($P \leq 0.05$) changes in these parameters. A low fracture stress value indicates a greater fracturability. Control cheeses fractured more easily than saffron cheeses, whereas S cheeses showed the highest fracture stress values. For control cheeses, fracture stress values slightly decreased until d 30 ($P \leq 0.05$), but after that period, the values started to increase until d 180 ($P \leq 0.01$). Saffron cheeses also increased fracture stress with time ($P \leq 0.01$).

Fracture strain describes the deformability of the cheese; high numerical values indicate higher deformability. Fracture strain decreased with ripening time ($P \leq 0.01$), meaning that cheeses were more elastic at the beginning of ripening period. Control cheeses were less elastic than saffron cheeses but as the ripening period lengthened, the differences between samples were less evident. Control cheeses showed the lowest deformability values.

Stress Relaxation. This test was done to study the viscoelastic response of cheese during ripening. Values for e and r were obtained, as explained in the Mate-

rials and Methods section. Values of r (s^{-1}) equal to zero correspond to a more elastic solid, whereas values near 1 indicate viscous behavior. Results are shown in Figure 2C and 2D. After manufacturing, cheese $3 \times S$ showed higher e -values that were different ($P \leq 0.01$) from the rest of the cheeses. Between d 15 and 60, no differences were found. After d 60, saffron addition had an influence on e -values, showing differences ($P \leq 0.001$) between saffron concentrations (i.e., as saffron concentration was increased, cheeses became more elastic). Values for r were not affected by saffron addition, except on d 3 when control cheeses had higher values ($P \leq 0.026$). Regarding ripening time, both parameters showed opposite behavior: whereas e decreased with ripening time, r increased, showing that ripening time influences elastic properties, resulting in less elastic cheeses at d 180.

Sensory Analysis

The purpose of the triangular test was to study if consumers were able to make differences between saffron and non-saffron cheeses during ripening. To accomplish the objective, cheeses with the lowest saffron concentration were given to consumers to be compared with the control. Color differences between the control and saffron cheeses were noticeable to consumers ($P \leq 0.05$) at all stages where sensory evaluation was conducted (60, 120, and 180 d). Flavor discrimination was also significant ($P \leq 0.05$) until the end of the ripening period.

The ranking test was carried out to know if consumers were able to rank from the lowest to the highest saffron concentration for color and flavor attributes.

Figure 2. Mean values of fracture stress (A), fracture strain (B), equilibrium residual values of normalized relaxation stress when time $\rightarrow \infty$ (e ; C), and elastic behavior (r ; D) in control cheese (□), specific concentration of saffron-added cheese (S; Berruga Fernández et al., 2009; ■), 2 × S (twice the concentration of S) cheese (○), and 3 × S (3 times the concentration of S) cheese (●) as a function of ripening time. Significance levels for each ripening time are indicated as follows: * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

Consumers were able to order saffron cheeses from the less yellow to the more yellow color at the 3 stages of ripening tested ($P \leq 0.001$). Significant differences were also found between cheeses with different saffron concentrations. Flavor ordering was more difficult than color ordering. At 60 d of ripening, flavor differences were statistically significant ($P \leq 0.001$), meaning that consumers were able to order the cheeses from the lowest to the highest saffron flavor. After this time, flavor differences between cheeses, although they were significant ($P \leq 0.05$), were less evident: only S cheeses were significant different from 3 × S cheeses.

DISCUSSION

Most of the compositional parameters tested did not show significant differences between control and saffron cheeses, with the exception of salt content and nitrogen fractions. Nevertheless, certain effects due to saffron addition can be observed, especially regarding bacteria counts, texture, and color.

Control cheeses presented slightly higher total and lactic acid bacteria counts than saffron cheeses, which probably caused a faster lactic acid production during pressing and, thus, lower pH values. Moreover, control cheeses presented higher WSN, SNpH4.6, and 12% TCA values than saffron cheeses, especially at 60 d of ripening, as a possible consequence of slightly higher lactic acid bacteria enzymatic activity. Results suggest that saffron could be slowing down the growth of these bacterial groups. Nevertheless, no studies, to our knowledge, have been published concerning the influence of saffron on lactic acid bacteria. A few studies have focused on the antimicrobial properties of saffron, confirming its antimicrobial properties in different bacteria [e.g., *Micrococcus*, *Staphylococcus*, *Escherichia coli*, *Salmonella*, *Helicobacter pylori*, and fungi (e.g., *Candida*, *Aspergillus*, and *Cladosporium*)], but only moderate activity has been found (Vahidi et al., 2002; Kamble and Patil, 2007; Sekine et al., 2007; Nakhaei et al., 2008; Pintado et al., 2011). Further research is needed to confirm saffron's effect on lactic acid bacteria found in saffron cheeses.

Regarding composition, DM content values in control cheeses were slightly lower, especially at d 3, whereas salt concentrations were significantly higher. As mentioned previously, faster lactic acid production could be taking place in control cheeses, which is directly related to pressing time. Pressing time is, in part, responsible for whey drainage and is dependent, in the case of some pressed cheeses, on pH. In control cheeses, pH decreased faster, so the pressing time was reduced from 5 to 4 h, resulting in higher water content at d 3. This higher water content could increase the capacity of exchange

between whey retained in the curd and salt, thus causing higher salt content in control cheeses. Higher salt content also stimulates proteolytic activity of rennet (Guinee and Fox, 2004); consequently, it might be causing the faster proteolysis rate at the beginning of ripening in control cheeses.

These differences among control and saffron cheeses regarding salt content, DM content, and proteolysis could be the origin of the textural differences found, as texture is developed by many interrelated factors, including the casein matrix, as well as fat, water, and pH. Texture results showed that control cheeses were firmer than saffron cheeses, which is given by the lower fracture stress values. Several authors have reported that firmness of cheese increases with decreasing water content during ripening, thus causing a loss of elastic structural elements (Juan et al., 2007). As mentioned above, control cheeses showed slightly lower DM content than saffron cheeses, explaining the firmness differences between them. Control cheeses were less deformable ($P \leq 0.05$) and elastic than saffron cheeses because they had less fracture strain and higher r values, especially at the beginning of ripening. At d 30, an inflection point in fracture stress of control cheeses was observed, as reported in other ewe milk cheeses (Pavia et al., 1999; Bertolino et al., 2011). This could be explained by the conversion of fresh curd into a mature curd at this stage or ripening, which is influenced by pH (O'Callaghan and Guinee, 2004). Watkinson et al. (2001) found a positive correlation between pH and fracture strain, confirmed by our results, as control cheeses had lower pH values.

Regarding ripening in general terms, control and saffron cheeses presented similar physicochemical values than those reported for pressed ewe milk cheeses (Pavia et al., 2000; Ballesteros et al., 2006; Cabezas et al., 2007). Dry matter, fat, protein and salt content increased during ripening, similar to changes found by Pavia et al. (1999). Nitrogen fractions in control and saffron cheeses were similar to those reported before; nevertheless, the values increased more slowly with ripening time, showing a slower rate of degradation of caseins (Guamis et al., 1997; Ballesteros et al., 2006; Cabezas et al., 2007). Our results showed that rennet was more active than starter bacteria, because the SNpH4.6 fraction had higher values than the 12% TCA fraction (i.e., soluble peptides produced by rennet were not hydrolyzed by bacterial peptidases at the same rate as they were produced; Gobetti, 2004).

As expected, color was the parameter that had evident changes, as saffron concentration increased because of the coloring properties of saffron. Color data for control cheeses proved to be in line with published results for hard cheeses (Rohm and Jaros, 1996), with the only ex-

ception that values for coordinate a^* in control cheeses were lower than those obtained by other authors in similar cheeses (Pavia et al., 1999). Cheese storage time and exposure to light have previously been studied in processed cheeses where L^* values decreased with storage time (Kristensen et al., 2001). This decrease was found to change linearly with time as a consequence of a browning reaction, probably nonenzymatic, leading to loss of brightness. Values of coordinate b^* for saffron cheeses were similar to Cheddar cheese shreds colored with annatto, whereas a^* was lower and L^* higher, which translates into a more yellow than red color (Colchin et al., 2001). Saffron color mainly evolves due to glycosylated esters of dicarboxylic acid, named crocetin esters, resulting in the yellowish red hues of the spice, so that increasing saffron concentration was reflected by coordinate b^* differences between saffron cheeses.

Coordinate L^* decreased with ripening time; the major changes in coordinates a^* and b^* were between d 3 and 15 and after this period remained almost constant. Our results agreed with studies on Emmental cheeses where L^* decreased, b^* increased, and a^* did not show a definite trend throughout ripening (Rohm and Jaros, 1996). These changes could be attributed to loss of water and, thus, fat concentration during ripening, especially changes in b^* , as the influence of fat on increasing yellow coloration of cheeses has been demonstrated (Rohm and Jaros, 1996).

Crocetin esters have been intensively studied regarding their coloring properties, but the influence on color of different liposoluble carotenoids that are also present in saffron, such as β -carotene, ξ -carotene, zeaxanthin, and lycopene, have not been thoroughly studied (Carmona et al., 2006). These molecules are prone to degradation due to light exposure, thermal treatment and acidic environment, having a direct impact on saffron coloring properties (Sánchez et al., 2008). Cheeses were exposed to air for 60 min, showing color changes, turning less bright, red, and more yellow as exposure time was increased. This loss of red coloration could be due to oxidation of liposoluble carotenoids present in saffron, whereas increments in coordinate b^* could be due to a concentration of crocetin esters due to loss of water on the surface. Previous studies found that decreasing water content in saffron favored the preservation of crocetin esters (Alonso et al., 1993) and avoided loss of yellow coloration.

Sensory evaluation showed color and flavor differences between cheeses. Sensory color differences proved to be in line with the colorimetric parameters obtained, as yellow coloration was the most affected by saffron concentration and was visually evident. During sessions, some panelists commented that saffron flavor was present in cheeses during the early stage of ripening,

but as the ripening period increased, this flavor was masked by the development of the characteristic cheesy flavor. Moreover, they thought that the cheese flavor was enhanced by saffron addition. This may explain the fact that flavor differences between cheeses were less evident as ripening time was increased. It is well known that saffron has been used since ancient times for its flavor properties and as a flavor enhancer, although this property has not been studied (Carmona et al., 2006); further research could be useful for this field of study.

CONCLUSIONS

Color was the main aspect modified by the use of saffron on pressed ewe milk cheeses. Saffron cheeses were less bright, less red, and more yellow than the control. Cheese color was also affected by air exposure, making it appear less bright, less red, and yellower with greater exposure time. Sensory analysis revealed that saffron modifies the color and flavor of cheeses and differences between saffron concentrations were perceived. The total and lactic acid bacteria counts were lower in saffron cheeses, suggesting that saffron could be slightly slowing down bacterial growth, causing a smaller pH decrease during pressing and, thus, increasing pressing time and DM content after salting. Saffron cheeses showed lower salt content and were firmer and more elastic. Slight differences in proteolysis rate were observed, although these differences were not evident by the end of the period studied. This work offers, for the first time, new insights about the influence of saffron on a pressed ewe milk cheese, revealing the need for further studies regarding antimicrobial activity and its properties as a flavor enhancer. This cheese is also an important approach to the diversity of traditional products.

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