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Characterization of artisanal saffron ricotta cheese produced in Sicily: Physicochemical, microbiological, sensory, and antioxidant characteristics

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ABSTRACT

The present study aims to characterize the artisanal saffron ricotta cheese produced from the whey of Piacentinu Ennese protected designation of origin (PDO) cheesemaking, including via technological parameters detected during the production process and by assessment of the main physicochemical, microbial, sensory, and antioxidant characteristics. A survey on the manufacture process of saffron and control ricotta cheese was conducted on 3 farms, located in the production area of the Piacentinu Ennese PDO cheese. pH and temperature followed a specific behavior, characterized by an inverse trend where pH decreased and temperature increased, playing an important role in the production process. All the analytical parameters were affected by the presence of saffron, also showing high between-farm variability, with significantly higher total solids and fat contents in saffron ricotta cheese compared with the control cheese (28.68% vs. 23.86%, and 19.83% vs. 14.22%, respectively). Microbial analysis showed significantly lower values in saffron compared with control ricotta cheese, for coliforms (1.51 vs. 1.91 $\log_{10}$ cfu/g, respectively), yeasts (1.55 vs. 2.06 $\log_{10}$ cfu/g, respectively), and molds (1.03 vs. 1.30 $\log_{10}$ cfu/g, respectively), denoting potential reduction of microbial growth asserted by saffron. *Escherichia coli* concentration (1.26 $\log_{10}$ cfu/g) in saffron ricotta cheese was in accordance with EU Regulation 2073/2005 and then safe for consumption. The presence of saffron influenced all sensory attributes, particularly color and aroma. Interestingly, high total antioxidant activity was found in saffron ricotta cheese (372 μC) compared with the control cheese. Thus, this artisanal dairy production could be considered a suitable option for functional foods with antimicrobial properties, due to the presence of saffron, which may contribute to extend the shelf life of the product. Further studies need to focus on the bioactive compounds that affect the antioxidant proprieties, characterization of the microbiota of saffron ricotta cheese, and evaluation of consumers' acceptance and perception as well as market demand.

Key words: saffron ricotta cheese, Piacentinu Ennese, functional foods

INTRODUCTION

In Sicily, in the Enna area, the dairy tradition enjoys a wide range of products, among which the Piacentinu Ennese protected designation of origin (**PDO**) cheese stands out, a compact pressed cheese made with whole raw sheep milk with natural fermentation acidity, characterized by more or less intense yellow color due to the presence of saffron used as an ingredient in the making process (European Commission, 2010).The resulting whey is used for ricotta cheese production. Moreover, according to the PDO production regulation, the scotta whey resulting from the ricotta cheese production is used for the second cooking of the curd in Piacentinu Ennese PDO manufacture (European Commission, 2010).

Ricotta cheese is a typical fresh dairy product, considered a by-product of cheesemaking, still made in an artisanal way in different Italian regions, including Sicily, where it represents one of the main dairy products (Mancuso et al., 2014; Mangione et al., 2023a). Saffron ricotta cheese is a peculiar production, with a yellowish color, that contains some saffron residues deriving from the production of the cheese. It is therefore a traditional saffron-based dairy product with a particular flavor and unique sensorial and aromatic characteristics (Gaglio et al., 2019).

Currently, most of the saffron ricotta cheese produced is sold under salt, as the sale is more difficult for the fresh product. Indeed, although it is the normal continuation of cheesemaking, only a few producers make this type of ricotta cheese. In Sicily, ricotta cheese production is an important source of income, amortizing the costs of the cheese plant; thus if it remains unsold, it represents a considerable loss for the producer.

According to producers, the main problem encountered in the sale of saffron ricotta cheese seems to be

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the straw yellow color, which is not appreciated by consumers, as the characteristic white color is usually associated with sheep ricotta cheese. Therefore, probably the consumer prefers not to buy the product only for an aesthetic issue without really knowing its organoleptic and sensorial characteristics.

Due to this lack of knowledge, it seems necessary to characterize this artisanal production, with natural saffron residues, relevant both for its qualities as an ingredient in several dishes and for its possible health beneficial effects. Literature reports several studies in which the nutritional characteristics of artisanal dairy products, including ricotta cheese, were increased by adding health-promoting ingredients such as aromatic plants and spices, including saffron, which are potential sources of natural bioactive components such as antioxidants, dietary fiber, and vitamins, preventing deterioration of the dairy products, and extending their shelf life (Hamdy and Hafaz, 2018). These components can produce new functional foods, exhibiting higher nutritional and therapeutic properties (Alenisan et al., 2017; Siyar et al., 2022) as well as possible alternatives for chemical preservatives and antimicrobial agents, preventing the growth of spoilage-causing microorganisms (Barak and Mudgil 2022).

Among spices, the effect of the use of saffron and its compounds (crocin, picrocrocin, safranal, etc.) in dairy productions has widely been studied in several researches. Saffron is the name of the spice derived from the red-dried stigmas of *Crocus sativus* L. flowers, and is one of the most expensive spices in the world, cultivated in many countries, including Italy; in Sicily it is mainly cultivated in the province of Enna (Branca and Argento 2009; Codurso et al., 2016). Saffron is well known for its beneficial effects on human health, preventing several diseases, as well as exerting antimicrobial activity against different bacteria, yeasts, and molds (Licón et al., 2010; Melnyk et al., 2010; Milajerdi et al., 2015).

In the traditional production of the Spanish whey cheese called requesón, the use of saffron and safranal, a saffron compound related to aroma, showed potential antifungal properties and reduced microbial growth during storage (Licón et al. 2012b; Librán et al., 2014). Recently Siyar et al. (2022) developed a new functional ricotta cheese by adding nanoliposomal encapsulated saffron extract, to obtain a product that is a source of bioactive compounds.

So far, no studies on Sicilian saffron ricotta cheese, made from Piacentinu Ennese PDO cheese production, are available in literature. The aim of this work was to characterize artisanal saffron ricotta cheese, including the detection of the technological parameters during the production process, and the assessment of the main physicochemical, microbial, and sensory characteristics, and the antioxidant activity of the product, compared with artisanal sheep ricotta cheese.

MATERIALS AND METHODS

No human or animal subjects were used, so this analysis did not require approval by an Institutional Animal Care and Use Committee or Institutional Review Board.

Experimental Plan

Three dairy farms (designated **A–C**) located within the Enna area, that produce both Piacentinu Ennese PDO and traditional Pecorino cheeses, 2 raw sheep milk cheeses, were selected both for the ricotta cheese sample collection and technological parameter detection during the saffron ricotta cheese manufacturing process. The 2 cheese productions differ only in the initial addition of saffron to milk before cheesemaking in Piacentinu Ennese PDO.

For each farm, samples of saffron ricotta cheese (**SRC**), produced with the whey derived from Piacentinu Ennese PDO cheesemaking, were collected. Also, control ricotta cheese (**CRC**), derived from the manufacture of the traditional Pecorino cheese produced in the same farms, was collected as a control trial. In each factory, ricotta cheese sample collections were run in triplicate: the first was carried out in February, the second in March, and the third in April 2022, within the production period of the PDO cheese.

All ricotta samples were transported under refrigeration conditions (4°C) to the laboratories of the Consorzio per la Ricerca nel settore della Filiera Lattiero-Casearia e dell'agroalimentare (CoRFiLaC, Ragusa, Italy) for analysis.

Ricotta Cheese Production and Sample Collection

Saffron ricotta cheese was produced using the whey derived from the Piacenitnu Ennese PDO cheese manufacture, whose production occurred in all the 3 factories with strict application of the protocol established for the protection of this traditional product from raw sheep milk, processed in wooden vats with the addition of local (Sicilian) saffron spice (European Commission, 2010). For the CRC manufacture, the whey derived from the traditional Pecorino cheesemaking was used.

For both SRC and CRC, the production process follows the same procedure reported by Mangione et al. (2023a). After curd separation, the resulting whey was filtered, placed into a large kettle, and heated to 45°C, and salt (0.8–1%) was added. Subsequently, raw sheep

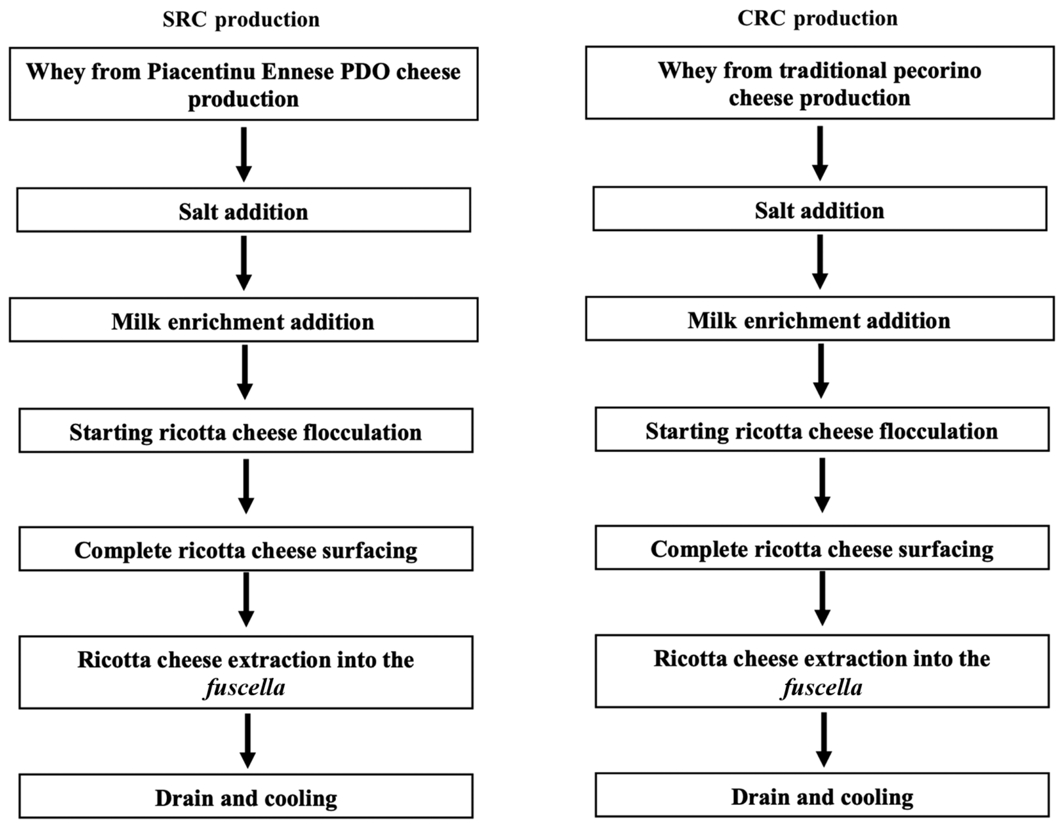

Figure 1. Flowchart of saffron (SRC) and control (CRC) ricotta cheese production. PDO = protected designation of origin.

milk (5–10%) was added at ~50°C. Afterward, the mixture was heated to ~85°C until the flocculated proteins rose to the surface. Once totally surfaced, ricotta cheese was manually collected and put into plastic cylindrical containers pierced to drain the scotta whey (baskets called *fuscelle*), left to drain at room temperature, and sampled 30 min after production. Production flowcharts for the artisanal SRC and CRC are shown in Figure 1.

Technological Parameters in SRC Production

A survey of SRC production practices applied in dairies A, B, and C, located in the Enna area, was carried out after PDO cheese production, to detect the technological parameters (pH, temperature) during the steps of production of the manufacture process (salt and milk addition, heat increase). Temperature and pH values during the entire production process were detected using a portable pH meter with a steel probe and an internal temperature sensor (Hanna-Instruments, Villafranca Padovana, Padova, Italy). All ingredients added during the production process were weighed. For a correct evaluation of the total weight loss of the ricotta cheeses, it was deemed necessary to weigh them after production, and 24 h after placing them into the *fuscelle*. Also, the kinetics of temperature and pH of SRC in postproduction were detected at 0 and 24 h.

Physicochemical Analysis

Two *fuscelle* for each ricotta cheese variety (SRC and CRC) for each farm were sampled to perform chemical analyses. After that, each ricotta was homogenized using Ultraturrax (Ultra-Turrax T 25 basic IKA-WERKE, Staufen, Germany).

Determination of pH was carried out with a pH meter MP 230 (Mettler Toledo, Switzerland) using an Inlab 413 combination electrode with integral temperature sensor. For TS content, samples were homogenized following the procedure described by Mangione et al. (2023a) and by drying the sample to a constant weight at 100 ± 1°C according to the American Public Health Association standard (APHA, 2004). The nitrogen and crude protein contents were measured by the Kjeldahl principle, using traditional and block digestion methods (ISO, 2014).

For fat content determination as a mass fraction of ricotta cheese, the Gerber-van Gulik method was used, in accordance with ISO 3433:2008 (ISO, 2008). Salt and ash contents were determined following the APHA

Standard Methods for the Examination of Dairy Products (APHA, 2004). All determinations were performed in duplicate.

Microbiological Analysis

Microbial analyses were performed in both SRC and CRC samples at 30 min (**T0**), 1 d (**T1**), 2 d (**T2**), and 5 d (**T5**) after production to monitor the microbial growth during the storage period at 5° ± 1°C, mimicking domestic storage conditions.

Selective groups of microorganisms were enumerated to study cheeses' microbial growth throughout cold storage. Ten grams of ricotta cheese sample was added to 90 mL of sterile (wt/wt) peptone salt solution and homogenized with a Stomacher Lab-Blender 400 (Seward Medical, London, UK) for approximately 5 min. In a sterile Ringer solution, 1:10 dilutions 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, 2013).

Total bacterial count (**TBC**) was enumerated on milk plate count agar (Liofilchem srl-Italy) after incubation at 30°C for 72 h aerobically, according to ISO (2013). For yeast and molds, yeast dextrose chloramphenicol agar (Liofilchem srl-Italy) incubated at 25°C for 5 d was used (ISO, 2004). Violet neutral red bile lactose agar (Liofilchem srl-Italy), incubated at 37°C per 24 h ± 2 h, was used as a solid selective medium for the enumeration of coliforms (ISO, 2006). *Escherichia coli* were enumerated on Tryptone-bile-glucuronide (Liofilchem srl-Italy) incubated at 44°C ± 1° per 24 h following ISO (2010). Finally, Baird Parker agar base (Liofilchem srl-Italy) was used as a selective basal medium for coagulase-positive staphylococci, to detect *Staphylococcus aureus*, according to ISO (2021). For all samples, the microbiological counts were carried out in duplicates at each collection time.

Sensory Analysis

The sensory characteristics of the SRC and CRC samples were evaluated after 24 h of production, through a quantitative descriptive analysis according to the ISO 13299:2016 protocol (ISO, 2016). The sensory analysis was carried out in individual computerized booths (UNI EN ISO 8589:2014; ISO, 2014) by 16 selected trained assessors, following ISO 8586:2012 (ISO, 2012). Each panelist was asked to score a set of 23 descriptive attributes grouped into 5 categories (appearance, aroma, smell, taste, and texture).

The tasting sessions were performed in duplicate using Compusense 5 v. 4.6 software (Compusense, 2003). Approximately 50 g of ricotta cheese at 16 ± 2°C in white cups, marked with a 3-digit code, in balanced and randomized order, were presented to the panelists. Evaluation of the samples was performed using a continuous linear scale from 1 to 10 (1 = absence of sensation, 10 = maximum intensity of sensation). Moreover, an entire sample of each ricotta cheese was also shown to the panelists for the evaluation of appearance attributes.

Antioxidant Capacity

The total antioxidant capacity (**TAC**) for both SRC and CRC samples was assayed using e-BQC Natural Ingredients, a very sensitive device (0.1533 e-BQC/μ*M*) with disposable strips that uses electrochemistry analytical technique to measure antioxidant capacity (Bioquochem, Oviedo, Spain). After finding the right factor of dilution, 80 μL of the diluted samples was tested and the results expressed as **e-BQC** values, representing the charge of the electrons released by the antioxidants present in the sample to neutralize free radicals. For each sample, the measurement was performed in duplicate.

The results obtained were expressed in micro-Coulomb (μC) To convert e-BQC values into equivalents of Trolox (**TEAC**) and vitamin C equivalents (**CEAC**, vitamin C equivalent antioxidant capacity), commonly used standards, calibration curves were performed with the device using Trolox and vitamin C as standards (Bioquochem kit), respectively.

Statistical Analysis

The obtained data were statistically analyzed with JMP 16 software (SAS Institute Inc., 2022) using a generalized linear mixed model, including time of storage only for microbial analysis, as well as farm (FM), ricotta cheese type (RIC), and the variable ricotta cheese type nested within the farm effect (FM[RIC]). For the sensory analysis, the effect of the assessors was also introduced into the model as a random effect. Means were compared using Student's *t*-test. Statistical significance was attributed to *P*-values <0.05.

RESULTS

Technological Parameters

The main ricotta cheesemaking technological parameters measured are reported in Table 1. From the data detected during the SRC making process, several steps can be distinguished, corresponding to different ingredient addition as well different temperature and pH values. Starting from the beginning (39.04°C), after a

Table 1. Technological parameters detected during saffron ricotta cheese production process

Parameter	Mean	SD	Time (min)
Whey quantity (L)	127.42	70.74	0
Whey temperature (°C)	39.04	2.19	
Whey pH	6.61	0.04	
Salt addition (%)	0.66	0.09	5
Salt addition temperature (°C)	45.70	6.44	
Salt addition pH	6.53	0.08	
Milk addition (%)	8.95	2.50	15
Milk addition temperature (°C)	53.53	4.14	
Milk addition pH	6.49	0.07	
Temperature of flocculation (°C)	78.18	3.00	55
pH of flocculation	6.31	0.14	
Temperature end of flocculation (°C)	80.10	1.59	65
pH end of flocculation	6.24	0.12	
Temperature of scotta whey (°C)	79.78	3.03	80
pH of scotta whey	6.35	0.24	
Temperature of ricotta cheese (°C)	74.30	3.93	95
pH of ricotta cheese	6.35	0.10	

thermal increase of 6.64°C of the whey in the vat at pH 6.53 ± 0.08, salt is added (0.66%, wt/vol). Afterward, at a temperature of 53.5 ± 4.14°C and pH 6.5 ± 0.07, ovine raw milk is added (8.9%, vol/vol).

After 40 min from milk addition, the whey protein gel floats to the surface at 78.18 ± 3°C and pH 6.31 ± 0.14 without any addition of acid. The end of flocculation takes place at 80.1 ± 1.59°C and pH 6.24 ± 0.12, and, after a brief pause for consolidation of the gel, the ricotta cheese is manually extracted and collected inside perforated plastic forms, called *fuscelle*, arranged on a steel flat to drain the scotta whey. After 30 min from production, the ricotta cheese temperature and pH were 74.3 ± 3.93°C and 6.35 ± 0.1, respectively.

From the data detected, the percentages of variation in weight indicate a loss for the SRC of about 23% from 0 to 24 h, related to the scotta whey drainage, and an increase in pH from 6.35 ± 0.1 to 6.55 ± 0.04.

Chemical Determinations

The chemical composition values of both SRC and CRC samples are reported in Table 2. Almost all the chemical parameters of the 2 cheese varieties were affected by the dairy farm effect, except for pH and NaCl. The SRC TS contents were in the range of 22.43 to 33 g/100 g and were significantly higher than those of CRC ($P < 0.01$).

The fat contents of both SRC and CRC in farms A and C showed significantly higher values ($P < 0.01$) than those of farm B. A similar trend was observed for SRC protein content, which was significantly lower in farm A ($P < 0.01$) compared both with the CRC and the SRC of the other farms.

Salt in the TS content of the ricotta cheese samples analyzed ranged from 1.72 to 2.83 g/100 g for SRC and 2.12 to 3.77 g/100 g for CRC. No significant differences ($P > 0.05$) were found among the examined samples.

Fat in the TS content of SRC was significantly higher in farm A than in the others, and the protein in TS was conversely significantly lower for farm A compared with the other farms, ranging from 16.15 to 37.24 g/100 g and from 26.91 to 37.82 g/100 g for SRC and CRC samples, respectively.

On the whole, SRC showed significantly ($P < 0.01$) higher TS and fat contents compared with CRC samples (28.68% vs. 23.86% and 19.83% vs. 14.22%, respectively). Significant differences were also found in ash ($P < 0.01$), NaCl/TS ($P < 0.0.5$), and protein/TS ($P < 0.001$) contents, with lower values in SRC compared with CRC. Regarding pH and protein values, no significant differences were found between SRC and CRC samples.

Microbiology

The microbial results of SRC and CRC are reported in Tables 3 and 4. The results indicated that all factors, the dairy farms, the time of storage, and the ricotta cheese type, are statistical factors in determining microbial composition. Particularly, significant differences were found between SRC and CRC for TBC, coliforms, and yeasts during the storage time investigated (T0, T1, T2, T5). These differences were only significant for specific days ($P < 0.05$).

The TBC in both ricotta cheese samples during the storage time followed a similar pattern of growth, ranging from 3 to 4 $\log_{10}$ cfu/g, and were lower in SRC at T0 and T5 compared with CRC (from 3.75 to 4.62 $\log_{10}$ cfu/g, and from 3.95 to 4.96 $\log_{10}$ cfu/g, respectively).

Coliforms and yeasts were lower in SRC than in CRC throughout the storage period. In particular, after 5 d

Table 2. Chemical composition of saffron ricotta (SRC) and control ricotta (CRC) cheeses, produced on 3 Sicilian farms (A–C); g/100 g, except for pH[1]

	FM[RIC]								RIC			
	A		B		C							
Parameter	SRC	CRC	SRC	CRC	SRC	CRC	SEM	P-value	SRC	CRC	SEM	P-value
pH	6.11	6.12	6.47	6.34	6.35	6.43	0.246	0.741	6.31	6.30	0.142	0.942
TS	30.62^{ab}	27.19^{bc}	22.43^{cd}	23.20^{cd}	33.00^{a}	21.19^{d}	1.841	<0.01	28.68^{a}	23.86^{b}	1.063	<0.01
Ash	0.98^{c}	1.34^{ab}	1.534^{a}	1.526^{ab}	1.02^{c}	1.32^{b}	0.067	<0.001	1.18^{b}	1.40^{a}	0.039	<0.01
NaCl	0.52	0.58	0.60	0.67	0.57	0.80	0.077	0.367	0.57	0.68	0.044	0.091
Fat	26.00^{a}	19.33^{ab}	9.67^{c}	11.50^{bc}	23.83^{a}	11.83^{bc}	2.734	<0.01	19.83^{a}	14.22^{b}	1.579	<0.05
Protein	4.92^{c}	7.78^{ab}	8.39^{ab}	8.64^{a}	6.70^{bc}	5.68^{c}	0.597	<0.01	6.67	7.37	0.345	0.179
NaCl/TS	1.72	2.12	2.83	3.06	1.76	3.77	0.461	0.0951	2.10^{b}	2.98^{a}	0.266	<0.05
Fat/TS	85.13^{a}	70.94^{ab}	43.38^{c}	48.76^{bc}	71.57^{ab}	56.09^{bc}	8.315	<0.05	66.69	58.60	4.800	0.256
Protein/TS	16.15^{c}	28.58^{b}	37.24^{a}	37.82^{a}	20.47^{c}	26.91^{b}	1.723	<0.0001	24.62^{a}	31.10^{a}	0.995	<0.001

[a–d]Means within row for each parameter with different letters were significantly different ($P < 0.05$; $P < 0.01$; $P < 0.001$; $P < 0.0001$).

[1]FM[RIC] = ricotta type nested within farm. RIC = cheese type.

of storage (T5), yeasts were significantly lower in SRC than in CRC cheeses ($P < 0.0001$).

No significant differences were found for water activity (**Aw**), molds, and *E. coli* during the storage period for either ricotta variety; however, molds and *E. coli* were slightly higher in CRC. The Aw remained constant during all storage periods for all samples.

Regarding the farm effect, significant differences ($P < 0.0001$) were found in farm C for both SRC and CRC samples, with lower values compared with the others (3.70 and 3.57 $\log_{10}$ cfu/g, respectively). Concerning coliform content, only CRC in farm A showed a significantly ($P < 0.01$) higher value compared with the other samples (2.63 $\log_{10}$ cfu/g).

Yeasts were significantly higher ($P < 0.01$) in farm A CRC sample than the rest (2.57 $\log_{10}$ cfu/g). However, molds were significantly lower ($P < 0.01$) in SRC samples than CRC for all 3 farms.

The ricotta type effect revealed that coliforms, yeasts, and molds were significantly lower in SRC than in CRC samples ($P < 0.05$ and $P < 0.01$, respectively). No significant differences were found between SRC and CRC for TBC, Aw, or *E. coli*, all of which showed similar values (Table 4).

Sensory Analysis

The sensory profiles of SRC and CRC are reported in Table 5. Both dairy farm and ricotta cheese type effects affected almost all sensory parameters. Among appearance attributes, only color uniformity showed no significant differences between the farms ($P > 0.05$), with similar values reached by both SRC and CRC samples in all 3 farms (score ranges 7.43–7.63 and 7.91–8.08, respectively). The SRC of farms A and B showed significantly higher ($P < 0.05$) color attributes compared with CRC values and farm C SRC. Lightness was significantly lower ($P < 0.0001$) in SRC compared with CRC in all farms. Structural uniformity was significantly higher in the farm A SRC sample ($P < 0.001$).

Regarding the effect of cheese type, all the appearance attributes were significantly different. In particular, SRC exhibited significantly greater color ($P < 0.0001$) and structural uniformity ($P < 0.05$) and significantly

Table 3. Microbial composition during storage of saffron ricotta (SRC) and control ricotta (CRC) cheeses, by day of storage (0, 1, 2, and 5)[1]

	SRC					CRC					
Item	0	1	2	5	SEM	0	1	2	5	SEM	P-value
TBC (30°C)	3.75^{c}	4.56^{ab}	4.42^{abc}	4.62^{ab}	0.557	3.95^{bc}	4.24^{ab}	4.12^{ab}	4.96^{ab}	0.560	<0.05
Coliforms	1.00^{c}	1.29^{bc}	1.97^{ab}	1.79^{ab}	0.630	0.97^{c}	2.10^{a}	2.56^{a}	1.89^{ab}	0.633	<0.01
Aw	0.98	0.97	0.98	0.98	0.003	0.99	0.98	0.97	0.98	0.003	0.06
Yeasts	1.11^{d}	1.45^{d}	1.45^{d}	2.19^{bc}	0.516	1.18^{d}	1.67^{cd}	2.40^{ab}	2.92^{a}	0.519	<0.0001
Molds	1.07	1.00	1.00	1.07	0.258	1.13	1.36	1.32	1.37	0.259	0.74
Escherichia coli	1.00	1.22	1.41	1.42	0.362	0.97	1.17	1.19	1.41	0.364	0.22

[a–d]Means within row for each parameter with different letters were significantly different ($P < 0.05$).

[1]Units are expressed in $\log_{10}$ cfu/g. TBC = total bacterial count. Aw = water activity.

Table 4. Microbial composition among Sicilian dairy farms (A–C) of saffron ricotta (SRC) and control ricotta (CRC) cheeses[1]

	FM[RIC]							RIC		
	A		B		C					
Parameter	SRC	CRC	SRC	CRC	SRC	CRC	P-value	SRC	CRC	P-value
TBC (30°C)	4.83^{a}	4.88^{a}	4.48^{a}	4.48^{a}	3.70^{b}	3.57^{b}	<0.0001	4.34	4.31	0.87
Coliforms	1.63^{b}	2.63^{a}	1.36^{b}	1.23^{b}	1.54^{b}	1.88^{b}	<0.01	1.51^{b}	1.91^{a}	<0.05
Aw	0.98	0.98	0.98	0.98	0.98	0.97	0.20	0.98	0.98	0.26
Yeasts	1.91^{b}	2.57^{a}	1.25^{c}	1.60^{bc}	1.50^{bc}	1.99^{b}	<0.01	1.55^{b}	2.06^{a}	<0.01
Molds	1.05^{b}	1.36^{a}	1.05^{b}	1.00^{b}	1.00^{b}	1.54^{a}	<0.01	1.03^{b}	1.30^{a}	<0.01
Escherichia coli	1.10	1.39	1.17	1.05	1.52	1.20	0.10	1.26	1.21	0.68

[a–c]Means within row for each parameter with different letters were significantly different ($P < 0.05$).

[1]Units are expressed in $\log_{10}$(cfu/g). FM[RIC] = ricotta type nested within farm. RIC = cheese type. TBC = total bacterial count. Aw = water activity.

lesser lightness ($P < 0.0001$) and color uniformity ($P < 0.0001$) compared with CRC.

Among odor attributes, only lactic acids were significantly affected by the farm effect ($P < 0.05$), and all the farms had lower scores ($P > 0.05$) in SRC than CRC for the other attributes. Considering the cheese type, odor attributes were significantly affected ($P < 0.001$), except for vegetable odor and aroma, which showed similar values between SRC and CRC (3.69 and 3.56, respectively). Lactic acid and cooked milk odors were significantly lower in SRC compared with CRC ($P < 0.001$), whereas the saffron odor was higher in SRC ($P < 0.001$), as expected.

Most of the taste attributes were significantly higher in SRC compared with CRC in all farms. No significant differences were found regarding acid and astringent taste attributes between farms. Spicy flavor showed no significant differences between farms ($P = 0.112$), reporting higher ranges of scores in SRC compared with CRC (4.40–4.77 vs. 1.30–1.89, respectively) due to the presence of saffron.

The cheese type effect showed significantly higher values in SRC for spicy and bitter attributes ($P < 0.001$) and lower sweetness ($P < 0.05$) compared with CRC. The other taste attributes showed similar values in both cheese varieties.

All aroma attributes confirmed the same trends as odor attributes for both effects. Spicy aroma was significantly higher in SRC than in CRC ($P < 0.0001$). With regard to texture, significant differences were found for all parameters between farms; in particular, farm B SRC reported lower adhesiveness ($P < 0.01$) than CRC and the cheeses from other farms, whereas farm A SRC showed lower spreadability ($P < 0.001$) and higher pastiness ($P < 0.001$) compared with the CRC and the cheeses from other farms. Granularity was higher in the SRC of farms A and B ($P < 0.0001$) compared with CRC. Similar to the chemical composition, even for sensory parameters of SRC samples, the large variability is probably related to the large variability in the making technologies adopted by farms.

On the whole, SRC showed only significant differences in granularity attributes, whereas all other parameters were similar between the 2 ricotta varieties.

Antioxidant Activity

Figure 2 reports the results of the TAC, as assessed using the different tests, including e-BQC, TEAC, and CEAC. In all the assays, the TAC mean values were higher in SRC (372 e-BQC, 321 μ*M*, 429 μ*M*) than in CRC (318 e-BQC, 270 μ*M*, 364 μ*M*) samples, probably related to the presence of saffron in the cheese. The e-BQC, TEAC, and CEAC values of SRC samples were in the ranges of 230 to 560 e-BQC, 184 to 501 μ*M*, and 256 to 657 μ*M* respectively. No significant difference in TAC between SRC and CRC samples was observed.

DISCUSSION

Technological Characterization

From the SRC cheesemaking it was possible to assess that important aspects must be considered in the mechanisms of production, including pH, temperature, and the addition of enrichment ingredients (Mangione et al., 2023b).

The pH value of the starting whey in the making process resulted in an optimal value, as reported by other authors (Mucchetti and Neviani 2006). The percentage of salt addition was in line with others artisanal ricotta cheesemaking, representing an important parameter in the manufacturing process. Salt affects the structure of protein and the protein-water interactions, influencing both solubility of proteins and their rates of thermal denaturation, to favor whey protein gel formation (Mangino 1984). Moreover, the higher cation concentration, due to the addition of salt to the whey, increases

Table 5. Sensory profiles, on a scale from 1 to 10, of saffron ricotta (SRC) and control ricotta (CRC) cheeses produced on 3 Sicilian farms (A–C)[1]

	FM[RIC]								RIC			
	A		B		C							
Attribute	SRC	CRC	SRC	CRC	SRC	CRC	SEM	P-value	SRC	CRC	SEM	P-value
Appearance												
Color	6.86^{a}	3.30^{c}	6.88^{a}	2.96^{c}	6.44^{b}	3.03^{c}	0.139	<0.05	6.72^{a}	3.10^{b}	0.086	<0.0001
Lightness	4.90^{d}	7.92^{a}	6.21^{c}	6.84^{b}	5.79^{c}	7.06^{b}	0.175	<0.0001	5.63^{b}	7.27^{a}	0.109	<0.0001
Color uniformity	7.57	8.08	7.63	7.91	7.43	7.95	0.126	0.649	7.54^{b}	7.98^{a}	0.078	<0.0001
Structure uniformity	6.73^{a}	5.52^{d}	6.04^{bcd}	6.41^{ab}	6.11^{bc}	5.78^{cd}	0.157	<0.005	6.29^{a}	5.91^{b}	0.121	<0.05
Odor												
Lactic acid	4.69^{b}	5.83^{a}	4.72^{b}	5.17^{b}	4.68^{b}	5.02^{b}	0.186	<0.05	4.70^{b}	5.34^{a}	0.116	<0.0001
Cooked milk	4.75	5.59	4.70	5.18	4.70	5.15	0.190	0.430	4.72^{b}	5.31^{a}	0.119	<0.0001
Vegetable	3.61	3.67	3.69	3.40	3.67	3.62	0.160	0.670	3.69	3.56	0.100	0.31
Saffron	5.65	1.26	5.42	1.51	5.10	1.59	0.181	0.126	5.39^{a}	1.45^{b}	0.112	<0.0001
Taste												
Spicy	4.77	1.30	4.63	1.66	4.40	1.89	0.187	0.112	4.60^{a}	1.62^{b}	0.117	<0.0001
Sweet	3.80^{b}	4.83^{a}	3.92^{b}	3.85^{b}	3.93^{b}	4.00^{b}	0.177	<0.001	3.88^{b}	4.23^{a}	0.110	<0.05
Salty	3.07^{b}	3.54^{a}	3.07^{b}	3.48^{a}	3.54^{a}	3.19^{ab}	0.134	<0.05	3.22	3.40	0.083	0.088
Acid	2.80	2.85	2.67	2.69	2.93	2.60	0.146	0.5044	2.80	2.71	0.091	0.433
Bitter	2.29^{ab}	1.52^{c}	2.54^{a}	2.11^{b}	2.11^{b}	1.75^{c}	0.114	<0.001	2.31^{a}	1.79^{b}	0.071	<0.0001
Astringent	4.78	4.87	4.67	4.69	4.61	4.28	0.163	0.0977	4.69	4.61	0.102	0.536
Aroma												
Lactic acid	4.50^{bc}	5.36^{a}	4.30^{c}	4.85^{b}	4.43^{bc}	4.56^{bc}	0.182	<0.05	4.41^{a}	4.92^{b}	0.113	<0.001
Cooked milk	4.50	5.40	4.26	4.96	4.49	4.88	0.184	0.1902	4.42^{b}	5.08^{a}	0.115	<0.0001
Vegetable	3.56	3.60	3.50	3.19	3.66	3.22	0.160	0.2842	3.57	3.34	0.099	0.055
Saffron	5.93	1.22	5.29	1.42	5.43	1.56	0.181	0.0506	5.55	1.40	0.113	<0.0001
Spicy	5.03	1.18	4.70	1.54	4.62	1.70	0.184	0.1029	4.78^{a}	1.47^{b}	0.115	<0.0001
Texture												
Adhesiveness	6.13^{abc}	5.99^{bc}	5.60^{c}	6.18^{ab}	6.61^{a}	6.42^{ab}	0.206	<0.01	6.11	6.19	0.129	0.613
Spreadability	6.42^{bc}	7.10^{a}	6.12^{c}	6.38^{bc}	7.12^{a}	6.81^{ab}	0.183	<0.0001	6.55	6.55	0.114	0.140
Granularity	4.07^{b}	3.10^{c}	5.56^{a}	4.44^{b}	2.93^{c}	3.09^{c}	0.199	<0.0001	4.19^{a}	3.54^{b}	0.124	<0.0001
Pastiness	6.22^{a}	5.56^{b}	5.36^{b}	5.83^{ab}	6.24^{a}	6.29^{a}	0.208	<0.001	5.94	5.89	0.130	0.769

[a–d]Means within row for each parameter with different letters were significantly different ($P < 0.05$).

[1]FM[RIC] = ricotta type nested within farm. RIC = cheese type.

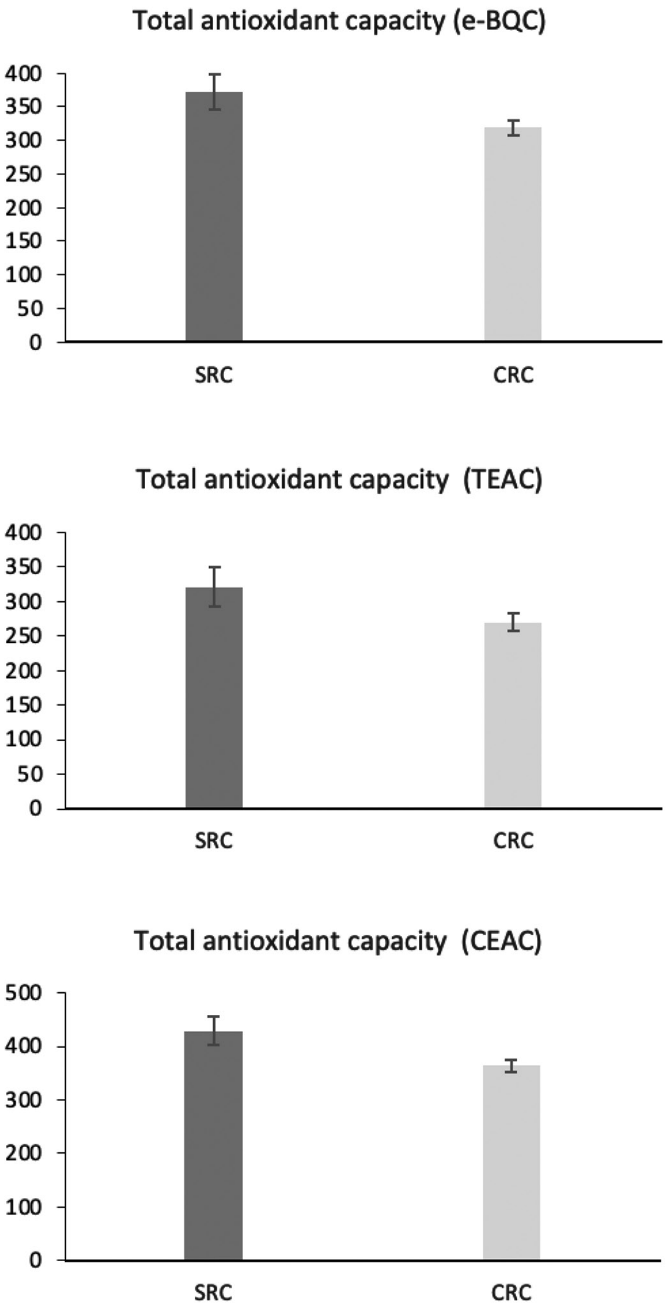

Figure 2. Total antioxidant capacity in saffron (SRC) and control (CRC) ricotta cheese samples. Error bars represent SD. e-BQC = charge of the electrons released by the antioxidants present in the sample to neutralize free radicals; TEAC = Trolox equivalents; CEAC = vitamin C equivalent antioxidant capacity.

the interactions with negatively charged proteins, thus improving whey protein aggregation (Mucchetti and Neviani 2006; Mucchetti et al., 2017).

Regarding the addition of whole milk, the temperature, as well as the quantity, were like those of other traditional ovine ricotta cheese (Filippetti et al. 2007). This step enriches the fat content of the whey and improves the sensorial and commercial characteristics of the ricotta cheese (Shahani, 1979; Mancuso et al., 2014), resulting in softer and creamier cheese with delicate texture (Pintado et al., 2001). Moreover, the addition of milk at 53.5 ± 4.1°C was aimed at preventing the residual rennet in the whey from degrading the casein (Weatherup 1986).

Similarly to other Italian artisanal ovine ricotta cheese production, in SRC manufacture it was not necessary to acidify whey to improve the protein aggregation, due to the natural pH value (Mucchetti et al., 2017). The changes of pH and temperature during SRC manufacture showed a specific behavior (Figure 3), characterized by an inverse trend where pH decreased while temperature increased, playing an important role in the making process. Both of these 2 parameters affect the formation and properties of whey protein gel (Mangino 1984). The heat during the making process influences both the rate of denaturation and the rates of protein interactions, balancing the rate of protein unfolding with that of aggregation. The pH markedly affects the structure of proteins and the amount of water that can be bound to proteins, influencing the proper balance between rate of denaturation and aggregation of the whey proteins, as well as the forces of attraction and repulsion between adjacent protein chains that are necessary to achieve a protein gel (Mangino 1984).

The method of production of the SRC highlighted a high degree of variability in the manufacturing process. In particular, the temperature of salt addition between farms showed a range between 40 and 55°C. Moreover for milk addition as well, different ranges for temperature and amount were detected (48–56.7°C and 5.6–11.83%, respectively), indicating that defining a general framework for production technologies is not easy, especially at an artisanal level.

Regarding the chemical composition, similar TS contents were found between SRC and other traditional sheep ricotta cheese (Filippetti et al., 2007; Spanu et al., 2017). The higher TS content observed in SRC compared with CRC could be related to the higher quantity of inorganic matter contained in the saffron (Mushtaq et al., 2015; Bhat et al. 2021).

Additionally, the fat content seemed to be affected by the presence of saffron, with a significantly higher value compared with the control sample, confirming results reported by other studies conducted in whey cheeses enriched with saffron (Licón et al. 2012b). The lower fat content in both samples from farm B was probably related to the milk and whey composition, whose physicochemical characteristics are affected by several parameters, such as the origin of the milk and whey (animal breed), animal feeding, and season of

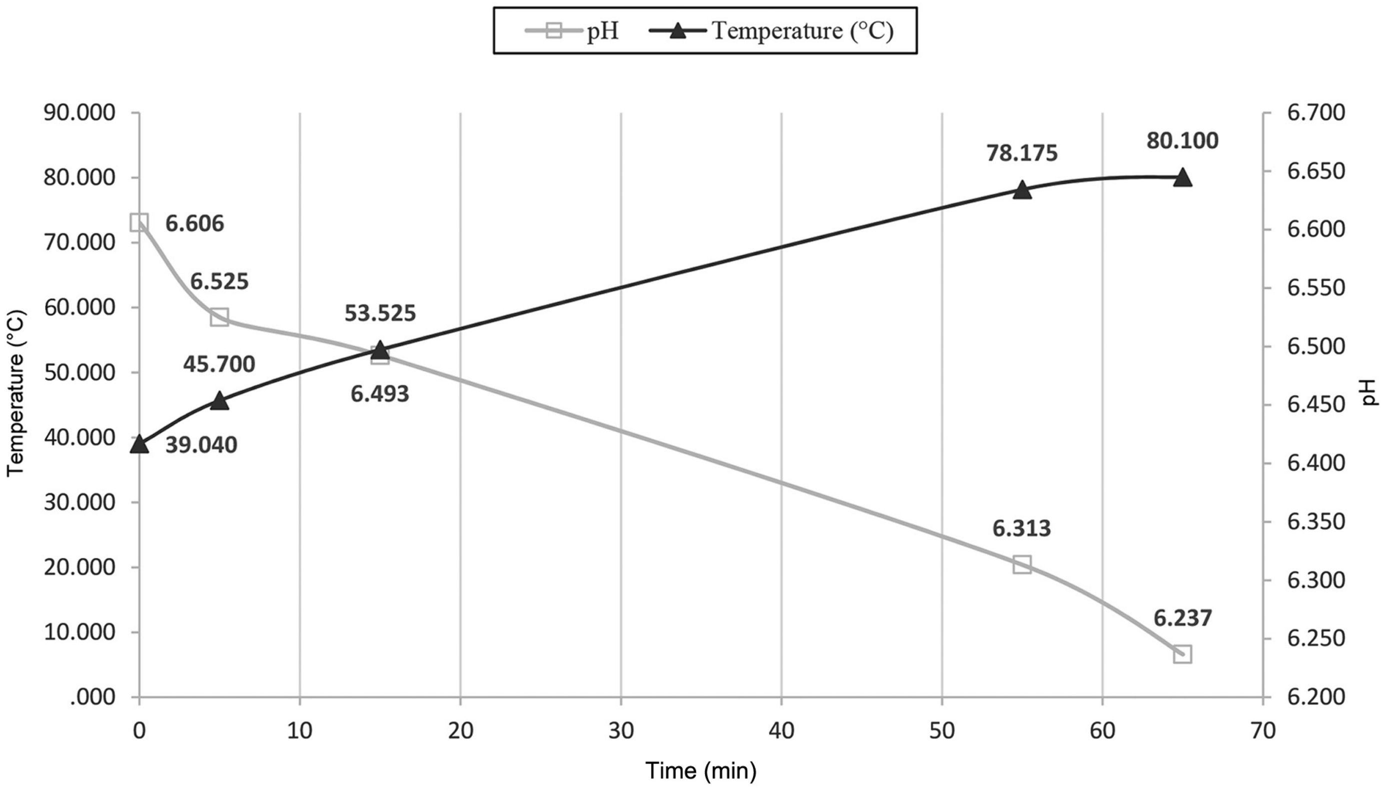

Figure 3. pH and temperature interaction during saffron ricotta cheese production.

production (Pizzillo et al., 2005; Mucchetti and Neviani 2006).

Ricotta cheese is a product that shows large variability in composition, due to the different characteristics and proportions of the dairy ingredients used (Mucchetti et al., 2017). The reason lies in the fact that it is not easy to control an exact production procedure from batch to batch and from farm to farm (Streiff et al. 1979).

The variability among producers, certainly usual in the case of artisanal products, is increased by the additional variability of the products during their manufacture. Indeed, despite the ricotta cheese production process applied following the same procedures across all the sampled farms, the differences in the product are mainly determined by the ingredients' composition and quality, as well as by the technology of production, including the amount of ingredient (percentage of milk used for the enrichment, salt percentage, and so on), different heat administration, and different timing.

All compositional values detected were within the range of other sheep ricotta cheese varieties reported in the literature (Mucchetti et al., 2002; Filippetti et al., 2007; Pianaccioli et al., 2007; Mancuso et al., 2014).

Microbial Growth Inhibition

Concerning the microbial features, results showed that all microbial groups were lower in SRC than in CRC for all the farms involved in this study. The differences observed between the farms regarding microbial growth could be related to several factors, such as the quality of raw material, respect of good production practices, and storage conditions (Ledenbach and Marshall, 2009).

Similar lower microbial growth trends have also been reported in other whey cheeses enriched with saffron (Licón et al. 2012b). It is also important to highlight that coagulase staphylococci were absent in both SRC and CRC during all storage days, and this was in accordance with the findings of Aktypis et al. (2018), who investigated an ovine cheese with saffron addition in which *Listeria* and *Staphylococcus* were never detected during storage.

All samples showed an *E. coli* concentration always lower than 2 $\log_{10}$ cfu/g, in the range of the detection limit for cheese made from milk or whey under heat treatment according to EU Regulation 2073/2005 (EC, 2005) and thus safe for consumption. These results indicate that saffron could be slowing the growth of some microbial groups in the dairy matrix, including in ricotta cheese. Indeed, because ricotta cheese has a high pH (above 6.0), high moisture content, high concentration of residual sugars, and absence of preservatives, it is a substrate very susceptible to microbial growth (Ricciardi et al., 2019).

Previous studies have focused on the antimicrobial and antifungal proprieties of saffron supplemented in dairy products, confirming its inhibiting proprieties on microbial growth (Licón et al. 2012a; Librán et al. 2014; Aktypis et al., 2018). The antimicrobial function of saffron can be attributed to its natural compounds, such as safranal and crocin, which are recognized for their antimicrobial activities (Pintado et al., 2011). These substances, due to their volatility and water-solubility, can easily reach the contaminant microorganisms, reducing and inhibiting their growth (Pintado et al., 2011).

According to Tortorello (2003), the counts of molds and yeasts can be used to assess shelf life of products. Therefore, from the results obtained from these microbiological parameters, it is possible to hypothesize that the presence of saffron could contribute to the conservation of SRC, reducing the deterioration of the product and extending the shelf life. However, the storage times considered in this trial may be too short (until 5 d) to draw conclusions regarding the differences between SRC and CRC samples ascribed to the presence of saffron. Possible expected evidence could be tested by extending the storage time. Thus, further research could be useful for this field of study.

Product Acceptability

As expected, color was the parameter that showed a higher score in SRC, due to the saffron coloring properties, in accordance with other studies that evaluated the sensory proprieties of dairy products enriched with saffron (Licón et al. 2012a; Gaglio et al., 2019) as well as the intense saffron odor, normally related to safranal, the major volatile compound of saffron (Rödel and Petrzika 1991). The lower sweetness and the consequent bitter taste of SRC were probably caused by picrocrocin, a monoterpene compound responsible for the bitterness in saffron (Lozano et al., 2000).

The SRC showed only significant differences in granularity attributes compared with CRC, whereas the other textural parameters were similar between the 2 ricotta varieties. Regarding the granularity, our results were inverse to those reported by other authors (Pizzillo et al., 2005; Fusaro et al., 2019), who correlated the higher parameter value to lower fat and higher protein contents. Conversely, in this study SRC showed higher fat and lower protein contents than CRC, with higher granularity. A possible explanation for this observation in the SRC samples could be due to the increased amount of inorganic matter, as well as the increase in the amount of fiber caused by the presence of saffron (Mushtaq et al., 2015; Bhat et al., 2021). Parameters were also confirmed by the chemical analysis, with a significantly higher ($P < 0.01$) TS content in SRC than CRC samples (28.68% vs. 23.86%, respectively).

Moreover, the low spreadability of SRC could be related to the lower moisture content of the product compared with CRC. Several authors, indeed, have reported that higher moisture resulted in higher spreadability (Miele et al., 2021; Rubel et al., 2022).

As for the other analyses, even for SRC samples, the artisanal production between-farm variability was reflected in sensory attributes (Fusaro et al., 2019; Miele et al., 2021).

Antioxidant Activity

In relation to antioxidant activity, the CRC showed TAC values 15% lower compared with the SRC samples, denoting a relevant, although limited, antioxidant activity, even without any source of bioactive ingredient (318 e-BQC). This could in part be linked to the animal diet available when the sample collection was carried out, composed of green pasture, which provides high levels of antioxidants (Chávez-Servín et al., 2018). Several studies have revealed that pasture contains many naturally bioactive molecules that affect the antioxidant activities of milk and dairy products (Jordán et al., 2010; Silanikove et al., 2010; Keles et al., 2017).

However, the evident higher TAC of SRC compared with CRC samples (372 vs. 318 e-BQC, respectively) is due to the presence of several potent bioactive compounds in saffron, such as crocetin, picrocrocin, and safranal (Singletary 2020). Previous studies have demonstrated that the addition of saffron in dairy matrices significantly increases the antioxidant activity of the products compared with controls, revealing the positive effect of this spice. Gaglio et al. (2019) demonstrated that the inclusion of saffron drastically increased antioxidant activity during storage, indicating that saffron fortification of yogurt results in a novel functional product that can be used as a complementary antioxidant source. Conversely, Aktypis et al. (2018) studied the effects of saffron supplementation in fresh ovine cheese, revealing a higher initial antioxidant activity that gradually decreased during its 30-d storage, without statistical differences compared with the corresponding value of control cheese.

However, regarding SRC production, it is important to specify that no saffron is added during the manufacture, but the residues of the spice are located in the whey, derived from the Piacentinu Ennese PDO cheesemaking, where it is possible to find most of the bioactive compounds added to the cheese (Bhat et al., 2021).

As a high antioxidant, saffron reflects its positive effects as a functional ingredient, natural colorant, shelf life enhancer, and fortifying agent in different food products, including dairy (Maqbool et al., 2022). Moreover, being an antioxidant that is derived from plants in the form of phenolic compounds, it exhibits high inhibition activity of lipid oxidation and a remarkable antimicrobial activity, which are of utmost importance to increase the shelf life of dairy products. (Gad and Sayd 2015; Aktypis et al., 2018).

Given all of the above aspects, and also considering the significantly lower microbial values detected in SRC samples during storage, saffron presence in ricotta cheese could improve the shelf life. Thus, the importance of the antioxidants contained in saffron is linked with both preserving the shelf life of the ricotta cheese by delaying the oxidation of polyunsaturated fatty acids and exerting beneficial effects for human health, increasing the consumption in diet. However further studies are needed to better clarify which bioactive components of saffron affect the antioxidant and antimicrobial proprieties of the ricotta cheese, increasing the shelf life and functional aspects.

CONCLUSIONS

Technological parameters in SRC manufacture revealed that the pH and temperature showed a specific behavior, with pH decreasing and temperature increasing during the entire production process, playing an important role in the making process. Chemical and microbial compositions were affected by the farm, whose differences mainly depend on the greater variability of the traditional manufacture. The sensory profile confirmed the higher variability between farms, revealing a remarkable yellowness color and saffron presence in aroma and taste attributes. The presence of saffron also affected the TAC, which was higher in SRC compared with control cheeses. This traditional production can be considered a suitable alternative option for functional foods, underlining an antimicrobial property that may contribute to extending the shelf life. However, further studies are required to identify which bioactive compounds affect the antioxidant proprieties, to characterize the microbiota, and to evaluate consumers' acceptance and perception of SRC as well as market demand.

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