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Microbiological and physicochemical profile of sugar added ewe's Ricotta during storage

Profilo microbiologico e fisico-chimico della ricotta di pecora zuccherata durante la conservazione

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Abstract

The microbiological and physicochemical traits of fresh ewe's Ricotta made with (40% w/w) and without sucrose were investigated. Sugar added Ricotta (SAR) and control Ricotta (CTR) were packaged and stored at 4°C for 90 d. *Salmonella* spp., *Listeria monocytogenes*, *Escherichia coli*, coagulase positive staphylococci, *Enterobacteriaceae*, total coliforms, *Pseudomonas*, yeasts, moulds and clostridia were undetectable during the entire monitoring. Total mesophilic and psychrotrophic microorganisms and mesophilic cocci lactic acid bacteria increased over time, but their development was limited in SAR production. The acidification was observed only for CTR. Water activity was consistently low (0.897 on average) in SAR that were also characterized by the highest percentage of dry matter (45.27 – 47.36%). Fat, protein and salt contents were in the range typical for ewe's Ricotta. The results provided evidences on a 40-d shelf life for SAR product.

Keywords:

- Cheese whey
- Ewe's Ricotta
- Microbiological stability
- Shelf life
- Sugar added Ricotta

Riassunto

Nel presente studio, sono state analizzate le caratteristiche microbiologiche e fisico-chimiche di ricotte fresche di pecora prodotte con (40% p/p) e senza aggiunta di zucchero. Sia le ricotte zuccherate (SAR) che quelle della produzione controllo (CTR) sono state confezionate e conservate a 4°C per 90 g. *Salmonella* spp., *Listeria monocytogenes*, *Escherichia coli*, stafilococchi coagulasi positivi, *Enterobacteriaceae*, coliformi totali, *Pseudomonas*, lieviti, muffe e clostridi erano sotto il limite di rilevabilità per l'intera durata del monitoraggio. I livelli dei microrganismi mesofili e psicrotrofi totali e quelli dei batteri lattici cocchi mesofili sono aumentati nel tempo, sebbene il loro sviluppo fosse più limitato nei campioni SAR. L'acidificazione è stata osservata

soltanto per le ricotte della produzione controllo. L'attività dell'acqua è risultata particolarmente bassa (in media 0.897) nelle ricotte zuccherate che hanno mostrato anche la percentuale più alta di sostanza secca (45.27 – 47.36%). Le percentuali di grasso, proteine e sali rilevate sono risultate in media con i valori generalmente riportati per la ricotta di pecora. Infine, i risultati hanno chiaramente mostrato che la shelf life della ricotta di pecora zuccherata è di circa 40 giorni.

Parole chiave:

- Ricotta di pecora
- Ricotta zuccherata
- Shelf life
- Siero
- Stabilità microbiologica

INTRODUCTION

Ricotta is a dairy product, manufactured from the whey residual from cheese production, but cannot legally be defined as a cheese. It derives from the coagulation and precipitation of whey proteins favored by pH decrease and heating at 80-90°C (1). In these conditions, lactalbumins and lactoglobulins solidify coming to the surface in the form of flakes which are collected and transferred into perforated plastic molds (2). Ricotta can be made from goat's, ewe's and cow's whey individually or combined (3).

In the last years, the demand for Ricotta increased consistently. This phenomenon is mainly related to the consumers' choice for healthy foods with high nutritional value. However, due to the high moisture level, a high concentration of residual sugars and an initial pH > 6.0, Ricotta constitutes an optimal substrate for the growth of several microorganisms. As a consequence, this product is highly perishable and its shelf life is particularly short (4). Even though the thermal treatment kills the majority of raw milk microbial populations and only a few thermotolerant bacteria may survive the high temperatures applied during production, Ricotta is susceptible to secondary contaminations that, generally, occurs during transport, storage and commercialization (5). Sardinia and Sicily are Italian regions where sheep milk is produced at consistent levels (6) and the main Ricotta produced and sold in the islands are made from ewe's whey. Traditional ewe's Sicilian Ricotta is produced enriching whey with whole raw milk (5-15%) and salt (0.5-1.5%) to increase yield and improve the organoleptic characteristics (1, 7). As a matter of fact, ewe's Ricotta represents an excellence of gastronomy in Sicily, where it is commonly used as a topping ingredient for dressing pastas and, especially to prepare desserts, rather than being consumed as a dairy product (8). Several Sicilian desserts and confectioneries like "cassata siciliana", "cannolo", "sfincia di San Giuseppe", "cassata al forno", "cassatelle", "cartoccio fritto palermitano", "iris fritta", etc. contain sugar added ewe's Ricotta as main ingredient (**Fig. 1**). The aim of this work was to evaluate the shelf life of sugar added ewe's Ricotta produced at industrial level strictly respecting the good hygienic practices (GHP) and packaged in plastic film. The classical Ricotta formulation obtained from the same whey was used as control trial. Microbiological and physicochemical parameters were monitored during the entire 90-d storage.

MATERIALS AND METHODS

Production of Ricotta and sample collection

Ricotta was produced at the industrial dairy factory Biopek snc located at Gibellina (TP, Italy) applying the traditional protocol (**Fig. 2A**). Briefly, ewe's whey (1,500 L) obtained after curd breaking and draining (average temperature 37 – 38°C) was poured into a AISI 304 stainless

Figure 1. Main Sicilian desserts containing ewe's Ricotta as main ingredient: **A**, cassata siciliana; **B**, cannolo; **C**, sfincia di San Giuseppe; **D**, cassata al forno; **E**, cassatella; **F**, cartoccio di ricotta; **G**, iris fritta.

steel tilting steam cooking vessel (Cappellotto V. snc, Sarcedo, Italy) and initially heated at 60°C. Bulk whey was added with 5% (v/v) pasteurized ewe's whole milk and kitchen salt (5 kg). The temperature was raised up to 82°C and 0.3 % (w/v) calcium and magnesium salts (Syd Sal, Sidex S.p.A, Cercola, Italy) were added. Once whey proteins coagulated they were collected from the surface and placed into perforated plastic molds for the control (CTR) production. Sugar added Ricotta (SAR) (**Fig. 2B**) was obtained from fresh Ricotta to which 40% (w/v) fine white sugar (Nordzucker, Braunschweig, Germany) was added. SAR was then passed through a smoothing-homogenizing machine mod. V5LLISC-L15-000-00 (Plastitalia Group, Borgo San Lorenzo, Italy) to obtain a homogeneous creamy product. Finally, both Ricotta formulations (100 g) were packaged into three-layer coextruded plastic film "sac à poche" bags (Implast srl, Palazzolo sull'Oglio, Italy), compliant to the Reg. CE 1935/2004 and 2023/2006 and Reg. UE 10/2011, hermetically sealed and stored at 4°C until 90 d. CTR and SAR productions were replicated independently in three consecutive weeks. Each bag represented an individual sample. Sample collection was performed at 0, 15, 30, 40, 60, 75 and 90 d. In each production day, two bags per sampling time were obtained to perform a duplicate technical repeat. Thus, six samples per collection time (two technical repeats for three production days) were analyzed.

Microbiological analyses

Fifteen grams of each sample of CTR and SAR, for all sampling times, were transferred into sterile plastic bags, added with 135 mL of 2% (w/v) sodium citrate and homogenized by stomacher (BagMixer® 400, Interscience, Saint Nom, France) for 2 min at the maximum speed. All homogenized samples were serially diluted (1:10) in Ringer's solution (Sigma-Aldrich, Milan, Italy) into 10 mL-volume test tu-

Figure 2. Flow diagram of Ricotta production. A, control production; B, sugar added Ricotta.

bes homogenized by vortex. Cell suspensions were analyzed for the following microbial groups: total mesophilic aerobic microorganisms (TMM) and total psychrotrophic microorganisms (TPM) by spread plating on skimmed milk Plate Count Agar (milkPCA) (Biokar Diagnostics, Allonne, France), incubated at 30°C for 48 h and at 7°C for 6-7 d, respectively; *Pseudomonas* spp. on *Pseudomonas* Agar Base (PAB) (Oxoid Microbiology Products, Thermo-Scientific, Milan, Italy) incubated at 22°C for 72 h; yeasts and molds on yeast extract dextrose peptone (YPD) supplemented with chloramphenicol (0.1 g/L), incubated for 2-7 d at 25°C; presumptive mesophilic and thermophilic lactic acid bacteria (LAB) cocci on M17 agar (Microbiol, Cagliari, Italy) incubated anaerobically for 72 h at 30°C and 44°C, respectively; presumptive mesophilic and thermophilic LAB rods on MRS agar (Microbiol) adjusted to pH 5.4 with 5 Mol lactic acid, incubated anaerobically for 72 h at 30°C and 44°C, respectively; total coliforms and *Enterobacteriaceae* on Violet red bile agar (VRBA) and Violet red bile glucose agar (VRBGA), respectively, both incubated at 37°C for 24 h. In addition, all samples were also analyzed for the presence of the main pathogenic microorganisms: coagulase-positive staphylococci (CPS) on Baird Parker (BP) supplemented with rabbit plasma fibrinogen (RPF)

(Oxoid); *Listeria* spp. and *Listeria monocytogenes* on *Listeria* selective agar base with SR0140E supplement (Oxoid); *Salmonella* spp. and *Escherichia coli* on Hektoen Enteric agar (HEA) (Microbiol). All plates were incubated at 37°C for 24 h. Furthermore, clostridia were also object of investigation on Reinforced Clostridial medium (RCM). Clostridium content was estimated by the most probable number (MPN) technique using a 3 x 3 scheme: undiluted samples and decimal dilutions were pasteurized at 85°C for min an inoculated into RCM tubes supplemented with 1.4% (v/v) Na-lactate (Sigma-Aldrich, Milan, Italy); after that, test tubes were sealed with 2% (w/v) water agar. Clostridia were incubated at 37°C for 7 d.

Physicochemical analyses

The values of pH of the different samples of Ricotta were determined by a Seven Compact model S220 pH meter (Mettler Toledo, Leicester, United Kingdom).

Total titratable acidity (TTA) was determined by titration on 10 g of each Ricotta sample with 0.1 N NaOH until the pink phenolphthalein end-point and expressed as mL of NaOH/10 g. Specifically, 10 g of Ricotta were added with 90 mL of distilled water and homogenized by stomacher as reported above. The homogenates were transferred into beakers, added with five drops of phenolphthalein (1% w/v in ethanol) and titrated. Water activity (a_w) of CTR and SAR samples was determined by the Rotronic Hygropalm HC2-AW (Rotronic AG, Bassersdorf, Switzerland).

Moisture, fat, protein, saturated fatty acid, residual dry matter and salt content were determined by FoodScan TM 2 Lab (Instrument number 91861723 - Foss Electric A/S, Hillerød, Denmark). This instrument is based on near infrared (NIR) technology (9).

All physicochemical measurements were carried out in triplicate.

Statistical analyses

Microbiological and physicochemical data were subjected to one-way variance analysis (ANOVA) using XLStat software version 7.5.2 for Excel (Addinsoft, New York, USA). The Duncan procedure and

Tukey's test was applied for pairwise comparison. Statistical significance was attributed to p values of $p < 0.05$.

RESULTS

Microbiological characteristics of Ricotta

The results of the microbiological monitoring of the two formulations of ewe's Ricotta (CTR and SAR), commonly produced in Sicily, are reported in **Fig. 3** only for the microbial groups above the detection limit. None of CTR and SAR samples revealed the presence of detectable levels of *Pseudomonas* spp., yeasts and molds, total coliforms, members of *Enterobacteriaceae* family, CPS, *L. monocytogenes*, *Salmonella* spp. and *E. coli* at any sampling time.

Just after production, all other microbial groups object of investigation were below the detection limit, but at the 15th day of refrigerated storage TMM, TPM and mesophilic LAB cocci were enumerated in CTR samples. At the same time, SAR samples displayed detectable levels of TMM and mesophilic LAB cocci only. At 15 d, the levels of TMM for CTR samples (3.81 Log CFU/g) were almost two orders of magnitude higher than those registered for SAR samples (2.07 Log CFU/g), while the differences were lower (almost one Log cycle) for the group of mesophilic LAB cocci. At the 30th day of monitoring, TPM appeared also in SAR samples, but their cell densities were lower than those found in CTR samples (2.71 and 4.02 Log CFU/g, respectively). In general, an increasing trend in bacterial levels for TMM, TPM and mesophilic LAB cocci was observed for both Ricotta productions during time until the 75th day of analysis. The levels of these three groups were not statistically different from those estimated at the end of monitoring (90 d). During the entire refrigerated storage the levels of TMM, TPM and mesophilic LAB cocci of SAR samples were always lower than those of CTR samples. From the 30th day on, CTR showed the presence of thermophilic LAB cocci (3.74 Log CFU/g) whose levels increased until 6.98 Log CFU/g at

the end of storage. LAB rods were detected in CTR samples at the 75th day and increased consistently in levels at 90 d, when they reached 5.05 and 6.52 Log CFU/g for mesophilic and thermophilic groups, respectively.

Determination of the physicochemical parameters of Ricotta

The physicochemical characteristics of Ricotta are reported in **Table 1**. The values of pH were comparable between CTR and SAR Ricotta for the first 15 d. After that, a continuous drop of pH was registered for CTR production until reaching 5.63 at the 90th day. Regarding SAR samples, pH remained almost constant until the 75th day and the pH recorded at the end of the monitoring was only slightly lower (6.28). Concomitantly to pH drop, from the 30th day on, TTA of CTR Ricotta

Figure 3. Microbial loads (Log CFU/g) of Ricotta samples during storage. **A**, total mesophilic microorganisms; **B**, total psychrotrophic microorganisms; **C**, mesophilic coccus LAB; **D**, thermophilic coccus LAB; **E**, mesophilic rod LAB; **F**, thermophilic rod LAB. Results indicate mean values and standard deviation of six plate counts (carried out in duplicate for three independent productions). Abbreviation: CTR, control production; SAR, Sugar added Ricotta. Different lowercase letters indicate significant differences on microbial concentrations according to Duncan's test between CTR and SAR for $p < 0.05$, while different uppercase letters indicate significant differences on microbial concentrations according to Tukey's test between the same sample (CTR or SAR) at the different days of refrigerated storage for $p < 0.05$.

Table 1. Physicochemical traits of Ricotta samples during storage.

Samples	Parameters								
	pH	TTA	a_w	Moisture	Fat	Protein	Salt	Saturated fatty acids	Residual dry matter
Day 0									
CTR	6.41 ± 0.03 ^a	2.15 ± 0.10 ^a	0.991 ± 0.002 ^a	67.29 ± 0.48 ^a	16.33 ± 0.74	6.69 ± 0.11	0.74 ± 0.16	10.66 ± 0.39	32.72 ± 0.48 ^b
SAR	6.40 ± 0.02 ^a	1.55 ± 0.20 ^b	0.898 ± 0.005 ^b	54.73 ± 0.31 ^b	nr	nr	nr	nr	45.27 ± 0.41 ^a
Day 15									
CTR	6.34 ± 0.02 ^a	2.30 ± 0.00 ^a	0.989 ± 0.003 ^a	67.97 ± 0.69 ^a	16.03 ± 0.77	7.10 ± 0.51	0.86 ± 0.13	10.59 ± 0.23	32.03 ± 0.69 ^b
SAR	6.38 ± 0.04 ^a	1.80 ± 0.10 ^b	0.897 ± 0.002 ^b	54.64 ± 0.52 ^b	nr	nr	nr	nr	45.36 ± 0.52 ^a
Day 30									
CTR	6.05 ± 0.01 ^b	3.61 ± 0.10 ^a	0.989 ± 0.005 ^a	69.54 ± 0.35 ^a	16.08 ± 0.36	7.95 ± 0.28	0.81 ± 0.11	10.41 ± 0.30	30.46 ± 0.35 ^b
SAR	6.43 ± 0.02 ^a	1.97 ± 0.10 ^b	0.897 ± 0.001 ^b	53.42 ± 0.30 ^b	nr	nr	nr	nr	46.58 ± 0.40 ^a
Day 45									
CTR	5.90 ± 0.04 ^b	4.33 ± 0.10 ^a	0.989 ± 0.003 ^a	67.98 ± 0.31 ^a	16.61 ± 0.38	7.70 ± 0.58	0.77 ± 0.13	10.73 ± 0.42	32.02 ± 0.21 ^b
SAR	6.41 ± 0.02 ^a	2.02 ± 0.20 ^b	0.898 ± 0.002 ^b	53.11 ± 0.64 ^b	nr	nr	nr	nr	46.89 ± 0.64 ^a
Day 60									
CTR	5.86 ± 0.02 ^b	5.55 ± 0.20 ^a	0.986 ± 0.004 ^a	69.58 ± 0.35 ^a	15.68 ± 0.29	7.99 ± 0.12	0.86 ± 0.16	10.04 ± 0.14	30.42 ± 0.35 ^b
SAR	6.39 ± 0.03 ^a	2.23 ± 0.20 ^b	0.897 ± 0.003 ^b	52.64 ± 0.97 ^b	nr	nr	nr	nr	47.36 ± 0.47 ^a
Day 75									
CTR	5.64 ± 0.01 ^b	6.27 ± 0.10 ^a	0.987 ± 0.002 ^a	69.41 ± 0.55 ^a	16.23 ± 0.92	8.04 ± 0.22	0.73 ± 0.11	10.43 ± 0.31	30.60 ± 0.55 ^b
SAR	6.39 ± 0.01 ^a	2.66 ± 0.10 ^b	0.897 ± 0.002 ^b	52.99 ± 0.44 ^b	nr	nr	nr	nr	47.01 ± 0.44 ^a
Day 90									
CTR	5.63 ± 0.03 ^b	5.41 ± 0.10 ^a	0.988 ± 0.001 ^a	70.98 ± 0.55 ^a	15.08 ± 0.92	7.55 ± 0.22	0.64 ± 0.11	10.03 ± 0.31	29.02 ± 0.68 ^b
SAR	6.28 ± 0.04 ^a	2.88 ± 0.00 ^b	0.897 ± 0.002 ^b	53.28 ± 0.53 ^b	nr	nr	nr	nr	46.72 ± 0.53 ^a

Results indicate mean values ± standard deviation of six determinations (carried out in duplicate for three independent productions). Data within a column followed by the same letter between the CTR and SAR at the same day of refrigerated storage are not significantly different according to Duncan test at $p \leq 0.05$. Abbreviation: CTR, control production; SAR, Sugar added Ricotta; TTA, total titratable acidity; a_w , water activity; nr, not reported

increased consistently until 6.41 mL NaOH/10 g at the 90th day. On the contrary, TTA of SAR samples increased slightly during time and the final value was 2.88 mL NaOH/10 g.

a_w was evaluated for both CTR and SAR productions. In both cases, a_w remained almost constant during storage with an average value of 0.988 and 0.897 for CTR and SAR Ricotta samples, respectively.

Table 1 reports also the compositional analysis of Ricotta samples. However, due to the interference of the high sugar content with some determinations the results of fat, protein, salt and saturated fatty acid percentages are not reported for SAR samples. CTR samples were characterized by a moisture content that increased over time

from 67.29 to 70.98 %. On the contrary, SAR samples, showing a consistently lower moisture content than CTR samples, were characterized by a decreasing moisture level (from 54.73 to 53.28 %).

DISCUSSION

Even though Reg. EC No 2073/2005 of European Commission does not set any contamination limit for *Enterobacteriaceae* in whey products, Ricotta are often analysed for this bacterial group and the subgroups represented by total coliforms and *E. coli*. Scatassa et al. (1) during a retrospective analysis on the hygienic quality of fresh Ricotta produced in Sicily from 2002 to 2016 revealed that *Enterobacte-*

riaceae were detected in 21% of the samples analysed while *E. coli* in 13%. The authors attributed these results to a post thermal treatment contamination, since *Enterobacteriaceae* and *E. coli* should not be able to survive the high temperatures applied during the heat treatment of the whey (10). This is also confirmed by a very low incidence of coliforms in Sicilian Ricotta (10^3 CFU/g in only one samples out of eight samples analysed) when modified atmosphere packaging (MAP) was applied (4). A similar trend was observed by Pala et al. (11) who analysed ewe's Sardinian fresh Ricotta finding detectable levels (3.09 Log CFU/g) of *Enterobacteriaceae* in only one sample produced by a single farm (out of three farms monitored) and subjected to MAP. The same sample was also positive for the presence of *Pseudomonas*. However, when smoking was applied to Ricotta, *Pseudomonas* were not detected (12). The exponential increase of the levels of TMM and LAB over time is generally observed in fresh cow's and ewe's Ricotta (4, 13). In relation to the different LAB groups, our data are in agreement with those reported by Mancuso et al. (4) who displayed lower levels of LAB rods and thermophilic LAB cocci than mesophilic LAB cocci in fresh ewe's Ricotta produced in Sicily. The lower levels of TMM, TPM and LAB detected during the monitoring of SAR are imputable to the presence of a high concentration of sucrose that reduces the availability of water (acts as an a_w depressor) for the bacterial development and causes an osmotic stress (14). Food matrix composition impacts the development of LAB (15). Although a_w is not reported as a particularly critical parameter for LAB development (16, 17), low a_w values are not favourable for the growth of LAB (18). For this reason, a_w was evaluated for both CTR and SAR productions. In both cases, a_w remained almost constant during storage with an average value of 0.988 and 0.897 for CTR and SAR samples, respectively. Similar values for fresh ewe's Ricotta were reported by Pala et al. (11) after application of MAP for 21 d of refrigerated storage. When 40% (w/w)

sugar was added a_w decreased of ca. 0.090 which is a consistent diminution of availability of water for the development of bacteria, explaining the high differences in terms of bacterial cell densities between CTR and SAR. Salts and sugars are solutes that bind water (19) limiting the microbial development (20). Casti et al. (21) reported an a_w in the range 0.973 – 0.978 in ewe's Ricotta subjected to a dry salting step ("Ricotta salata") and stored for 90 d.

The more rapid drop of pH in CTR samples is due to the quicker development of LAB than in SAR samples. In general, pH decrease is related to the growth of LAB and their lactic acid production (22). TTA is correlated linearly with pH, since a decrease of pH corresponds to an increase of the mL of NaOH (23). The slow growth of LAB in SAR resulted in a low TTA kinetics while the increase in TTA registered for CTR samples was due to the production of organic acids produced by LAB whose cell densities increase during storage (24).

A moisture content above 70% is generally reported for ewe's Ricotta (11, 25). The lower moisture content revealed in Sicilian ewe's Ricotta in comparison with those produced in Sardinia is imputable to a higher percentage of whole milk added during Ricotta processing in Sicily. In fact, in Sicily milk addition to whey can be even 15% (v/v) (1), while 1.5 – 2% addition is generally performed in Sardinia (11). As expected, the residual dry matter was estimated at higher levels in SAR. Similar levels (around 45%) of total solids are also reported for salt added Ricotta salata (42.13 – 44.65%) (21). Regarding the content of fat, protein and salt, generally measured by other authors analyzing fresh ewe's Ricotta, there are several differences with the works available in literature, but these differences are not only imputable to the percentage of full fat milk addition, because also sheep breeds, season, environmental temperature, age, nutrition, stage of lactation and other factors influence the chemical composition of milk (26, 27) and, as a consequence, that of Ricotta (28).

CONCLUSIONS

The present work confirmed that ewe's Ricotta is a highly perishable product with a short shelf life. Traditional ewe's Ricotta produced in Sicily is characterized by a pH around 6.4 and a high a_w that represent intrinsic factors favorable for the development of a wide range of microorganisms. In general, the product obtained in small dairy factories that does not undergo a proper packaging can be commercialized within 4 – 6 d, but a packaging applied soon after production together with GHP and maintenance of refrigeration temperatures determined a marketability of fresh Ricotta at around 15 – 20 d. Even though chemical composition did not greatly change over time, microbiological data and acidification kinetics show-

ed that, at the 30th day, CTR production overcame the marketability deadline. Due to the relevance of sugar added Ricotta for the Sicilian confectionery industry and for the production of Sicilian dessert outside Sicily, this study analyzed also the characteristics of SAR during storage. In this case, the addition of 40% (w/w) sucrose determined a stability of all parameters, but especially the bacterial counts indicated that the shelf life cannot be longer than 40 d when TMM and LAB were comparable with those registered at 15 d for CTR production. The absence of process hygiene indicators and food safety microorganisms indicated the respect of the Regulation EC No 2073/2005 on microbiological criteria for foodstuffs during the production of both Ricotta formulations.

CONFLICT OF INTEREST DISCLOSURE

All authors declare that they have no conflict of interest inherent the present paper

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