PAPER

CHEMICAL AND MICROBIOLOGICAL CHARACTERISTICS OF HOMOGENISED RICOTTA CHEESE PRODUCED FROM BUFFALO WHEY

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ABSTRACT

To extend the shelf life of buffalo Ricotta cheese, a process was assessed that included a second heat treatment followed by homogenisation and hot packaging. The microbiological and chemical characteristics as well as the oxidation degree of the product were determined over storage for 21 days using total antioxidant activity, redox potential, malondialdehyde content, and protein-bound carbonyl content. Homogenised buffalo Ricotta cheese has a longer shelf life than traditional Ricotta cheese, although the process could be optimised to reduce the total bacterial load during storage. No significant oxidative damage occurred during storage. This innovative process could promote the market expansion of Ricotta cheese.

Keywords: antioxidant activity, carbonyl groups, malondialdehyde, oxidation level, Ricotta cheese, shelf life

1. INTRODUCTION

Ricotta cheese is an Italian dairy by-product obtained from whey via the denaturation of whey proteins at 80-85°C. In Italy, almost all families purchase Ricotta cheese at least once a year (ASSOLATTE, 2018). The consumption of Ricotta cheese is favoured by its low price. Despite its lower commercial value with respect to that of other cheeses, the whey proteins in Ricotta cheese have a high biological value owing to their significant content of sulphur-containing amino acids (SMITHERS, 2008).
Ricotta cheese has a high moisture content and an initial pH above 6.0. Its shelf life varies according to the treatment applied after curd floating and the type of packaging (MUCCHETTI and NEVIANI, 2006). Fresh buffalo Ricotta cheese is marketed locally and mainly consumed within 1-2 days of production. Its shelf-life is about 4 days (ZOTTOLA, personal communication).
The fact that the guaranteed shelf life of this product is only a few days hampers its distribution to distant markets. Current markets absorb only a small amount of fresh buffalo Ricotta cheese in comparison to the actual production potential, indicating that a large proportion of the buffalo whey deriving from Mozzarella cheese manufacture is not destined for Ricotta cheese but disposed of or destined for non-dairy use. An increase in the shelf life of buffalo Ricotta cheese could lead to an increase in the market share; this would in turn lead to a reduction in the quantity of whey to be disposed of.
In addition, the recent recognition of the Protected Designation of Origin (PDO) to "Ricotta di Bufala Campana" could increase interest from domestic and foreign markets in a product with a longer shelf life.
Shelf life can be increased by reducing microbial contamination, improving the hygiene conditions of the cheese plant equipment and environment, and reducing the cooling time (OTTOGALLI *et al.*, 1981; MUCCHETTI and NEVIANI, 2006). Furthermore, packaging systems, such as vacuum packaging, can extend the shelf life of Ricotta cheese (PINTADO and MALCATA, 2000). Modified atmosphere (MA) packaging has been used to extend the shelf life of fresh Ricotta cheese by up to 14 days (MANCUSO *et al.*, 2014).
A system that ensures a longer shelf life for Ricotta cheese is heat packaging. After the whey is drained, Ricotta cheese is heat-packaged into suitable containers (MUCCHETTI *et al.*, 2002). Additionally, after the whey is drained, Ricotta cheese can be subjected to a second thermal treatment and a homogenisation treatment. The homogenisation process occurs at a low pressure and inhibits product syneresis. Then, the homogenised Ricotta cheese is heat-packaged in sealed plastic containers (MUCCHETTI *et al.*, 2002). The heat packaging is applied to cow Ricotta cheese at an industrial level and is less often applied to Ricotta cheese from other species, or in small- and medium-sized cheese plants.
Other product innovations in the food chain have been introduced to meet new consumer needs. These include changes in the manufacturing process, product composition, packaging, and product size and shape, and the introduction of a new method of using the product (LIPAN *et al.*, 2017). Some examples of innovation in Ricotta cheese have been described by SCARANO *et al*. (2019).
To fulfil the needs of the buffalo dairy industry, the present study was designed to improve the technology of small plants to extend the shelf life of buffalo Ricotta cheese. A homogenisation of traditional Ricotta cheese preceded by heat treatment was suggested. Homogenised buffalo Ricotta cheese, obtained as described above, differs in sensory properties from traditional buffalo Ricotta cheese. Homogenisation produces a fine and uniform consistency with consequently greater creaminess (WILBEY *et al.*, 2012).

There have been many studies regarding the chemical and microbiological characteristics of Ricotta cheese (MUCCHETTI and NEVIANI, 2006; MUCCHETTI *et al*., 2017), but few have focused on its oxidative characteristics (RAIA *et al*., 1996). The double heating treatments and homogenisation applied to buffalo Ricotta cheese induced us to study the oxidation degree of this type of product at different times of storage.
In dairy products, lipids are susceptible to oxidation, a biochemical process that contributes considerably to the degradation of the nutritional and sensory qualities of a product during manufacture and storage; this causes a significant reduction in shelf life (Bergamo *et al*., 1998). This alteration in lipids is determined not only by the absorption of oxygen by both free and esterified unsaturated fatty acids but also by other environmental factors, including exposure to light, high temperatures, and contact with metals (Fe, Cu, Co, Ni, and Mn) (MORTENSEN *et al*., 2004).
Protein oxidation, another type of oxidative damage in dairy products that affects the protein matrix during heat treatment, generally induces changes in amino acid residues and three-dimensional protein structures and may result in the loss of biological functionality (AUGUSTYNIAK *et al*., 2015; FENG *et al*., 2015).
The aim of this study was, therefore, to evaluate the microbiological and chemical characteristics of homogenised buffalo Ricotta cheese over storage for 21 days. Moreover, the degree of oxidation of the product was investigated by adapting analytical methods to specifically study the Ricotta cheese matrix.

2. MATERIALS AND METHODS

2.1. Process of Ricotta cheese

The trials were performed in a medium-sized cheese plant located in Italy in the Lazio region where buffalo Mozzarella and traditional Ricotta cheese are produced using an artisanal system. The raw material used to produce traditional Ricotta cheese utilises the sweet whey drained from Mozzarella curd. Other important phases of the process are reported in Fig. 1.
Approximately 1.5% (w/v) fresh cream from buffalo whey was added to the sweet whey. Then, approximately 0.3% (w/v) NaCl was added, and the whey mixture was heated continuously in a large open kettle by direct heating. When the whey grains began to float, *sieroinnesto* (natural whey cultures obtained from previous cheese making) was added at various amounts according to the extent of the *sieroinnesto* titratable acidity (2-3% v/v). The heating of the mixed whey was stopped at 85°C, and when the firm curd floated to the surface, it was collected into perforated hoops where the whey was drained. The Ricotta cheese was held for one hour at room temperature and then transferred to a cold room at 4°C.
While the traditional Ricotta cheese was cooling, the temperature, pH, and weight were measured.
To produce fresh Ricotta cheese with increased shelf life, the following process (Fig. 2) was applied.

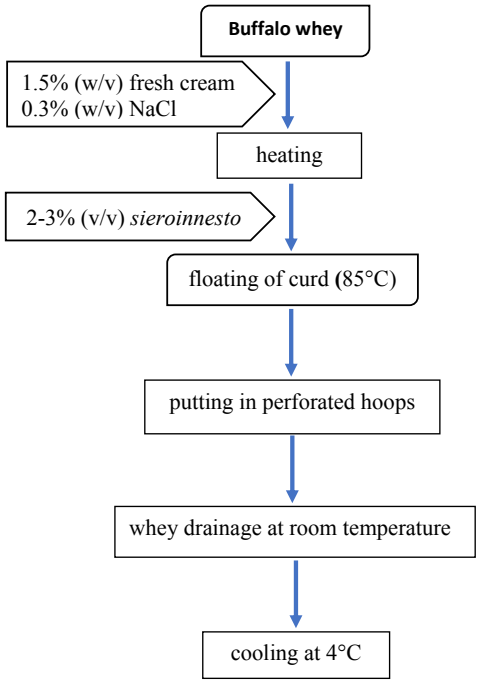

Figure 1. Flow-chart for the manufacture of traditional buffalo Ricotta cheese.

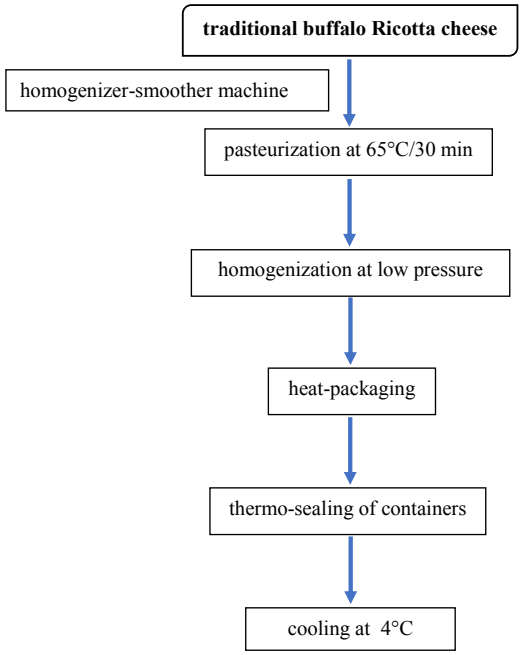

Figure 2. Flow-chart for the manufacture of homogenised buffalo Ricotta cheese

After draining, the traditional Ricotta cheese was transferred to a homogeniser-smoother machine. In this machine, which was equipped with a heating system, the Ricotta cheese was pasteurised at 65°C for 30 min and then homogenised at 15 bar.
Preliminary trials were carried out to determine the optimal initial moisture content of the product, pasteurisation parameters, and packaging conditions. The homogenisation process occurred at a low pressure. Finally, the product, namely homogenised buffalo Ricotta cheese, was immediately heat-packaged in sealed 250 g plastic containers to reduce spoilage risk, and then cooled in a room at 4°C.

2.2. Analysed samples

Sixteen samples were collected per day on three different days during production. Hermetically sealed 250 g Ricotta cheese packages arrived at the laboratory in refrigerated conditions and were stored at refrigeration temperature (4°C) for a period of 1, 7, 14, and 21 days.
Microbiological analyses were carried out on the refrigerated samples at the Animal Prophylaxis Research Institute for Lazio and Toscana Regions (IZSLT), Latina, Italy.
Chemical and oxidative analyses were carried out on frozen samples at the Council for Agricultural Research and Economics, Research Centre for Animal Production and Aquaculture of Monterotondo, Rome, Italy. For each sample, two Ricotta cheese packages were allowed to defrost overnight at 4°C. The sample was adequately homogenised, and the subsamples were taken for analyses. For each analysis, at least two replicates for each sample were performed.

2.3. Microbiological analyses

Bacteria in the fresh cheese samples were detected and enumerated in accordance with international standard methods. An initial suspension was prepared to achieve a uniform distribution of the sample microorganism content. The initial suspension was made by adding 225 ml of diluent to 25 g of sample for bacterial detection, and 90 ml of diluent to 10 g of sample for bacterial enumeration.
The samples were subjected to detection methods for *Salmonella* spp. (UNI EN ISO 6579-1:2017), *Listeria monocytogenes*, and *Listeria* spp. (UNI EN ISO 11290-1:2017).
Salmonella spp. were detected via pre-enrichment in buffered peptone water (BPW) broth incubated at 36±2°C for 18 h, enrichment in Rappaport-Vassiliadis soya peptone (RVS) broth and Muller-Kauffmann tetrathionate-novobiocin (MKTTn) broth at 37°C for 24 h, and plating on xylose lysine deoxycholate (XLD) agar and Salmonella-Shigella (SS) agar plates incubated at 37°C and examined after 24 h.
Listeria monocytogenes and *Listeria* spp. were detected via incubation in Half-Fraser broth at 30°C for 25±1 h, followed by a second enrichment with Fraser broth at 30°C for 25±1 h, which was then plated on Listeria agar, according to OTTAVIANI and AGOSTI (ALOA), and Oxford agar (LSM), and incubated at 37°C for 48 h.
β-glucuronidase-positive Escherichia coli was detected by incubating samples in tryptone-bile-glucuronic medium (TBX) at 44±1°C for 24 h (ISO 16649-2: 2001).
Coagulase-positive staphylococci (*Staphylococcus aureus* and other species) were detected using rabbit plasma fibrinogen agar medium incubated at 37±1°C for 24 h (UNI EN ISO 6888-2: 2004).
Pseudomonas spp. were detected by culturing the samples on penicillin and pimaricin agar (PPA) at 25°C for 48 h (ISO/TS 11059:2009- IDF/RM 225:2009).

Enterobacteriaceae was detected by culturing the samples on violet red bile glucose agar (VRBG) at 37°C for 24 h (UNI EN ISO 21528-2: 2017).
Yeasts and moulds with greater than 0.95 water activity were detected by incubating the samples on dicloran-rose bengal chloramphenicol agar (DRBC) at 25±1°C for five days (ISO 21527-1: 2008).
Total mesophilic counts (TMCs) were enumerated by incubating the 3M™ Petrifilm™ Aerobic Count Plate at 30±1°C for 72±3 h (AFNOR 3M 01/1-09/89).
Total psychrophilic counts (TPCs) were enumerated by incubating the 3M™ Petrifilm™ Aerobic Count Plate at 6.5±1°C for ten days (AFNOR 3M 01/1-09/89; ISO 6730: 2005 IDF 101).
Presumed mesophilic and thermophilic cocci were enumerated on M17 agar after aerobic incubation for 48 h at 30°C and at 44°C, respectively. Presumed mesophilic lactobacilli were enumerated on DeMan, Rogosa, and Sharpe (MRS) agar adjusted to pH 5.4 and incubated anaerobically at 37°C for 72 h. The result of bacteria enumeration was expressed as the number of microorganisms per gram of product.
Activity water (Aw) was determined with an Aqualab Lite (Decagon) principle dew-point measurement (ISO18787:2017).

2.4. Physical and chemical analyses

Homogenised Ricotta cheese samples were submitted to the following analyses: pH, moisture (IDF, 1986), total nitrogen (TN) (FIL-IDF, 1993), pH 4.6 soluble nitrogen (pH 4.6 SN) (FIL-IDF, 1991), fat (FIL-IDF, 2001), ashes (AOAC, 2000), and salt (IDF, 1988).

2.5. Antioxidant activity - the DPPH method

The antioxidant activity of the samples was evaluated using the DPPH (1,1-diphenyl-2-picryl-hydrazyl radical) method, as reported by Unal (2012), for milk and cheese, with some modifications. The purple stable free radical DPPH changes to a yellow colour following reduction by antioxidant molecules. This method is commonly used for the evaluation of the antioxidant capacity of plant extracts and food in various contexts (KATALINIC *et al.*, 2006; NIKOLOVA *et al.*, 2011).
Ricotta cheese samples (2 g) were mixed with 8 ml of 0.11 mM DPPH ethanolic solution. The control was prepared by adding 2 ml of ethanol to 8 ml of 0.11 mM DPPH ethanolic solution.
The mixtures were shaken vigorously and then left standing at room temperature for 20 min in the dark. Then, the mixtures were centrifuged for 10 min at 9000 g at 22°C.
The absorbance of the supernatant at 517 nm was measured using a double-beam UV-VIS spectrophotometer (Lambda 25, PerkinElmer). Absolute ethanol was used as a blank, and analyses were performed in duplicate.
The antioxidant activity was expressed as the percentage of inhibition of the DPPH radical according to the following equation:

$$\% \text{ antioxidant activity} = (A0 - As) / A0 \times 100$$

where A0 is the absorbance of the control (containing all reagents except Ricotta cheese sample),
and As is the absorbance of the tested sample.

Trolox was used as a standard to compare the antioxidant activity of the sample with a reference antioxidant. The antioxidant activity of the samples was expressed in Trolox equivalents (mmol Trolox eq. /100 g).

2.6. Redox potential determination

For each sample, the redox potential was determined using a potentiometric method that uses a pH meter (Metrohm 827 pH Lab) equipped with a platinum electrode (combined Pt-ring 6.0451.100) and the potential was expressed in millivolts (mV).

2.7. Malondialdehyde analysis

The method used for MDA determination was based on the detection of MDA - thiobarbituric acid (TBA) fluorescent complexes, in which the MDA present in a sample reacts with TBA, and the MDA-TBA complex results in an absorption peak at 532-535 nm (RAHARJO and SOFOS, 1993).
Ricotta cheese samples were prepared according to the methods of RAIA *et al.* (1996), with some modifications. A total of 0.4 g of the sample was homogenised in a solution of 3.4 ml of 10% w/v trichloroacetic acid (TCA) to precipitate proteins, and 0.20 ml of BHT (butylhydroxytoluene) 2.8% w/v ethanolic solution was added as an antioxidant to prevent further lipid peroxidation.
The mixture was heated at 90°C for 30 min, quickly cooled in an ice bath for 20 min, and centrifuged at 10,000 g for 10 min to separate the pellet containing the precipitated protein fraction.
A supernatant aliquot (300 µl) was added to 700 µl of 0.28% w/v TBA and incubated at 90°C for 30 min to induce the formation of the TBA-MDA complex, and the aliquot was then quickly cooled on ice for 20 min. After centrifugation at 10,000 g for 5 min, the supernatant was collected for the following HPLC analysis.
HPLC analysis was performed using a Shimadzu-SPD-M10A HPLC with a fluorimeter (RF-10A) and ZORBAX Eclipse Plus C18 column (4.6 x 250 mm x 5 μm). The isocratic mobile phase consisting of 5 mM sodium phosphate buffer (pH 7.0) and acetonitrile (80:20 v:v) was filtered on Whatman 0.2 μm filter paper. Aliquots (20 µl) were injected and analysed using a flow rate of 1 ml/min at room temperature. The fluorescent detector was set at an excitation wavelength of 515 nm and emission at 543 nm.
The concentration of the MDA-TBA complex was then calculated based on the calibration curve using a standard solution, and the values obtained were expressed as nmol/g.

2.8. Carbonyl determination

To evaluate the oxidative damage of proteins in the Ricotta cheese samples, the method of REZNICK and PACKER (1994) modified by FEDELE and BERGAMO (2001) for milk and cheese was used. The method is based on the derivatization of the carbonyl group with di-nitrophenol hydrazine (DNPH), and the chromophore produced can be detected at 360 nm.
In detail, Ricotta cheese samples (2 g) were weighed, and 5 ml of 0.2 M sodium citrate-NaOH (pH 8) at room temperature was added. After vigorous mixing for 5 min, 100 μl aliquots were incubated in the presence of 400 μl of 10 mM DNPH in 2.5 M HCl (in duplicate). In total, 400 μl of 2.5 M HCl (in duplicate) was added to the other 100 μl aliquots as a control (blank).

After incubation for 30 min in the dark at room temperature, 500 μl of cold 20% w/v TCA was added to each sample, and the samples were vigorously shaken. The mixtures were incubated for 20 min on ice and centrifuged for 10 min at 10,000 *g* at 4°C to cause protein precipitation.
The supernatant was removed, and 400 μl of 10% TCA was added to the pellet. After incubation for 10 min in the dark, the samples were centrifuged at 10,000 *g* for 10 min, after which the supernatant was removed.
To remove the free DNPH, the pellet was washed twice with 1 ml of ethanol/ethyl acetate (1:1 v/v) solution by centrifugation at 10,000 g for 10 min, and the supernatant was removed without disturbing the precipitate.
Pellets containing protein precipitates were then dissolved in 1 ml of 6 M guanidine HCl in 10 mM phosphate buffer (pH 2.3). The samples were incubated for 30 min in a 60°C water bath, and subsequently the absorbance was measured at 370 nm using a double-beam UV-VIS spectrophotometer (Lambda 25, PerkinElmer).
For the samples treated with 2 M HCl (control without DNPH), the protein concentration was determined by measuring the absorbance at 280 nm. The amount of protein was calculated from a bovine serum albumin (BSA) standard curve (0.5-2.5 mg/ml). The net absorbance was calculated as the difference between the absorbance of the sample with DNPH and the blank (without DNPH).
The concentration of protein-bound carbonyls (C Carb) was calculated by the molar extinction coefficient of 22,000/M/cm, according to the following formula:

$$\text{C Carb (nmol/mg prot)} = (\Delta \text{ Abs } 360 \ / \ 22 \text{ mM}) * 1000/\text{C prot}$$

where Δ Abs 360 = Abs 360 (DNPH) - Abs 360 (blank) is the net measured absorbance, and C prot is the protein content (g/L). The carbonyl concentration, expressed as nmol/mg of protein, was used to provide a global protein oxidation index.

2.9. Statistical analysis

All microbiological data were log-transformed. The GLM procedure of SAS software (SAS Institute Inc., 2007) was used for the statistical analysis of the chemical and oxidative analyses of Ricotta cheese. A factorial model, including the fixed effect of the storage time of Ricotta cheese, was used. The CORR procedure of SAS software (SAS Institute Inc., 2007) was also used.

3. RESULTS AND DISCUSSION

3.1. Process of making traditional buffalo Ricotta cheese

The initial titratable acidity of the whey transferred to the Ricotta cheese kettle ranged from 0.16 to 0.18 g of lactic acid/100 ml. ADDEO and COPPOLA (1983) found similar values of titratable acidity (0.17 g of lactic acid/100 ml) of starting whey from buffalo Mozzarella used for the manufacture of Ricotta cheese.
The process specifications of PDO "Ricotta di Bufala Campana" require a sweet whey with titratable acidity <0.16 g of lactic acid/100 ml. According to True (1973), in general, the initial titratable acidity of sweet whey destined to produce Ricotta cheese, should be less

than 0.16 g of lactic acid/100 ml. Optimal values are considered 0.13-0.14 g of lactic acid/100 ml.
In the traditional process of making buffalo Ricotta cheese, approximately 1.5% (w/v) cream was added to the whey.
According to "Ricotta di Bufala Campana" specifications, the addition of up to 6% (w/v) milk and 5% (w/v) fresh cream is allowed. The addition of milk or cream enriches the fat content of the whey and improves the sensorial characteristics of the Ricotta cheese (SHAHANI, 1979). Ricotta cheese manufactured with the addition of milk or cream is softer and creamier and has a delicate texture (PINTADO *et al.*, 2001). Fresh cream made from Mozzarella whey transfers a particular flavour to Ricotta cheese.
To enhance the coagulation and rise of the curd, *sieroinnesto* was added to hot whey. According to MUCCHETTI *et al.* (2017), in ovine Ricotta cheese production, it is not necessary to reduce the whey pH to favour protein aggregation, while cow and buffalo whey needs to be slightly acidified for better protein aggregation. Even the *sieroinnesto* addition can concur with a particular Ricotta flavour.
The amount of salt added to the whey (0.3%, w/v) was minimal with respect to the maximum amount allowed by "Ricotta di Bufala Campana" specifications (1%, w/v). NaCl dehydrates the whey proteins and has a destabilizing effect on BSA (FARKYE, 2004).
The industrial process of Ricotta cheese production (FARKYE, 2004) involves the stages described below. The whey is first neutralised to pH>6.5 (6.9-7.1) with a NaOH solution. Manipulation of the pH minimizes protein aggregation and produces a more cohesive coagulum (MODLER and EMMONS, 1989). The recommended temperature for milk addition (5-25%) is 65-70°C, while cream is added at 75-80°C. After the addition of cream, NaCl (0.5, v/v) is added. Then, an acetic or citric acid solution is added for coagulation and curd formation. Optimal coagulation and maximum yield occur at pH 5.6-5.8 (WEATHERUP, 1986).
In our trials, after three hours of cooling, the temperature of the Ricotta cheese decreased on average from 65°C to 17°C. The weight stabilised after three hours starting from the transfer to the basket. Weight loss for the traditional Ricotta cheese was approximately 15%. The final weight of the shapes was 400-450 g. After three hours, the pH increased from 6.22 to 6.76, while in Ricotta cheese from sheep's milk, the final pH ranged from 6.35 to 6.85 (CHERCHI *et al.*, 1999; SALVATORE *et al.*, 2014).

3.2. Process of homogenising buffalo Ricotta cheese

Regarding the optimal initial moisture of the product, according to preliminary trials, better results were achieved when the moisture content ranged from 67-70%.
The homogeniser-smoother machine used to produce homogenised Ricotta cheese was equipped with scraping blades, which stirred the product during heating. If the curd was too dry, the stirring was more difficult, and the curd adhered to the heating surface, reducing the effectiveness of the heat exchange. Moreover, the increase in temperature for low-temperature or batch pasteurisation (65°C/30 min) applied to heat the product was very slow.
Homogenised Ricotta cheese was heat-packaged, and to minimise microbiological pollution, the containers were thermo-sealed immediately after being filled. In the preliminary trials, we observed that the application of these conditions contributed to extending the shelf life of the homogenised Ricotta cheese. In fact, not sealing the containers immediately after filling them resulted in substantial contamination, particularly with mould and yeasts, after a few days (data not shown).

3.3. Microbiological characteristics

Salmonella spp. and *Listeria monocytogenes,* pathogens considered as food safety criteria (Reg CE 2073/2005), were not detected (below the detection limit), in accordance with the above regulations. In addition, *Listeria* spp. were not detected.
The counts of ß-*glucuronidase*-positive *Escherichia coli* and *coagulase*-positive staphylococci (*Staphylococcus aureus* and other species) are considered hygiene markers (Reg CE 2073/2005) and were lower than the detection limit of the method <10 colony forming units (cfu)/g (Table 1).

Table 1. Microbiological characteristics of homogenised buffalo Ricotta cheese during storage.
TMC: Total mesophilic count; TPC: Total psychrophilic count. Bacteria values are means±sd of three batches samples, expressed as log 10 cfu/g.

	Storage time			
	Day 1	**Day 7**	**Day 14**	**Day 21**
***ß-glucuronidase-positive Escherichia coli* (log 10 cfu/g)**	< 1	< 1	< 1	< 1
***Coagulase-positive staphylococci* (log 10 cfu/g)**	< 1	< 1	< 1	< 1
***Enterobacteriaceae* (log 10 cfu/g)**	< 1	< 1	< 1	< 1
***Pseudomonas* spp. (log 10 cfu/g)**	< 1	< 1	< 1	< 1
Yeasts (log 10 cfu/g)	< 1	< 1	< 1	< 1
Moulds (log 10 cfu/g)	< 1	1.30 ± 0.43	2.00 ± 1.41	< 1
TMC (log 10 cfu/g)	4.57 ± 0.41	6.42 ± 0.87	7.37 ± 0.47	8.27 ± 0.20
TPC (log 10 cfu/g)	2.52 ± 2.15	6.88 ± 1.45	7.35 ± 0.44	7.44 ± 0.90
Mesophilic lactococci (log 10 cfu/g)	4.34 ± 0.32	4.94 ± 0.30	7.24 ± 0.29	8.56 ± 0.63
Thermophilic lactococci (log 10 cfu/g)	4.31 ± 0.01	3.02 ± 2.86	7.20 ± 0.23	8.50 ± 0.71
Mesophilic lactobacilli (log 10 cfu/g)	2.72 ± 0.18	< 1	< 1	< 1
Aw	0.995 ± 0.001	0.995 ± 0.001	0.994 ± 0.0014	0.993 ± 0.002

Additionally, the counts of *Enterobacteriaceae* and *Pseudomonas* spp., which are often associated with changes in the texture and colour of the cheese, were lower than the detection limit (<10 cfu/g).
Yeasts were <10 cfu/g, while moulds were present only in one sample at day seven and in another at day 14. Moulds were within the limits according to ISO 21527-1: 2008. *Penicillium* spp. was identified through macroscopic and microscopic traits.
These results show that in the homogenised buffalo Ricotta cheese made by the process described above, pathogens are undetected, and all microorganisms considered hygiene markers were lower than the detection limit.
Our results agree with those reported by PALMAS *et al*. (1994) during the storage of sheep Ricotta cheese subjected to direct hot packaging.

The average TMC of our samples increased from 4.57 on day 1 to 8.27 log10 cfu/g on day 21. The same trend was observed for TPC, changing from 2.52 on day one to 7.44 log10 cfu/g on day 21. Mesophilic and thermophilic lactococci increased from 4.34 and 4.31 log10 cfu/g on day one to 8.56 and 8.50 log10 cfu/g on day 21, respectively. Mesophilic lactobacilli were present only in day one samples, and thermophilic lactobacilli were not detected (Table 1).
According to MUCCHETTI *et al.* (2002), industrially produced fresh Ricotta cheese, and particularly Ricotta cheese subjected to a second heat treatment, had a lower total bacterial count compared to that found in our samples.
Lactic acid bacteria are the microorganisms most commonly represented in Ricotta cheese, and they are an important part of the total bacterial count (MUCCHETTI *et al*., 2017). The increase during storage in TMC and LAB could be due to heat resistant or post-contamination microorganisms, since the high pH and elevated A_w do not limit microbial growth.
We assume that the microbiological characteristics of the product can be improved by the stricter control of environmental contaminants through the implementation of hygienic conditions during the process.
Furthermore, to reduce the number of heat-resistant microorganisms, higher temperatures could be used during the second heat treatment. Total bacterial count was lower during the storage of the product in industrial Ricotta cheese subjected to second heat treatment by 85-95°C (MUCCHETTI *et al*., 2002).
Moreover, an essential strategy for the control of mesophilic bacteria, such as spore-forming, is the reduction of cooling times and the strict maintenance of the cold chain during product storage.

3.4. Chemical characteristics

The chemical characteristics of the homogenised Ricotta cheese are reported in Table 2.

Table 2. Chemical characteristics of homogenised Ricotta cheese from buffalo whey.

Sample number	Moisture (%)	Dry matter (%)	Protein (%)	Soluble protein (%)	Fat (%)	Ash (%)
1	67.05	32.95	6.39	0.07	19.42	2.22
2	64.29	35.71	5.41	0.06	24.50	2.07
3	65.18	34.82	5.08	0.09	23.59	1.75
Mean	65.51	34.49	5.63	0.07	22.50	2.01
s.d.	1.41	1.41	0.68	0.01	2.71	0.24

The mean moisture content of the samples analysed was 65.51%, which was similar to that of the "Ricotta di Bufala Campana" cheese (64.58%) reported previously by several Authors (MUCCHETTI and NEVIANI, 2006). The moisture content of industrial Ricotta cheese from bovine and sheep whey is higher, 75.72% and 70.03%, respectively (MUCCHETTI *et al.*, 2002), both of which are made in large cheese plants where the

industrial process favours better whey recovery in the product than the traditional process (Salvadori DEL PRATO, 2001; MUCCHETTI *et al.*, 2002).
The mean protein and fat contents of homogenised buffalo Ricotta cheese were 5.63% and 22.50%, respectively, and the fat: protein ratio was 4.0. The samples analysed had lower protein and higher fat contents than the values found in "Ricotta di Bufala Campana" PDO, which were 9.43% and 19.09%, respectively (MUCCHETTI and NEVIANI, 2006). However, owing to the artisanal processing conditions, the data cited above are very variable: the protein content ranged from 7.10% to 13.46%, and the fat content ranged from 15.87% to 20.78%. The average composition of industrial cow milk Ricotta cheese is very different with 9.73% fat content, 9.12% protein content and a fat: protein ratio of 1.07 (MUCCHETTI and NEVIANI, 2006).
The mean value of pH 4 soluble protein was 0.07%, similar to that observed by MUCCHETTI *et al.* (2002) in Ricotta cheese from bovine whey at 0.10%. These data indicate the content of undenatured whey protein in Ricotta cheese. The use of other techniques such as ultrafiltration can increase the recovery of whey protein in Ricotta cheese (MAUBOIS and KOSIKOWSKI, 1978).
The mean ash content value was 2.01%, which was higher than that of "Ricotta di Bufala Campana" and cow Ricotta cheese (MUCCHETTI and NEVIANI, 2006); their values were 1.23% and 1.16%, respectively.

3.5. Physicochemical and oxidative characteristics

The physicochemical and oxidative characteristics of the homogenised Ricotta cheese from day 1 to 21 are reported in Figures 3, 4, 5, and 6. During product storage, the values of all characteristics were not significantly different.
The pH values (Fig. 3) decreased during storage from 6.90 to 6.55.

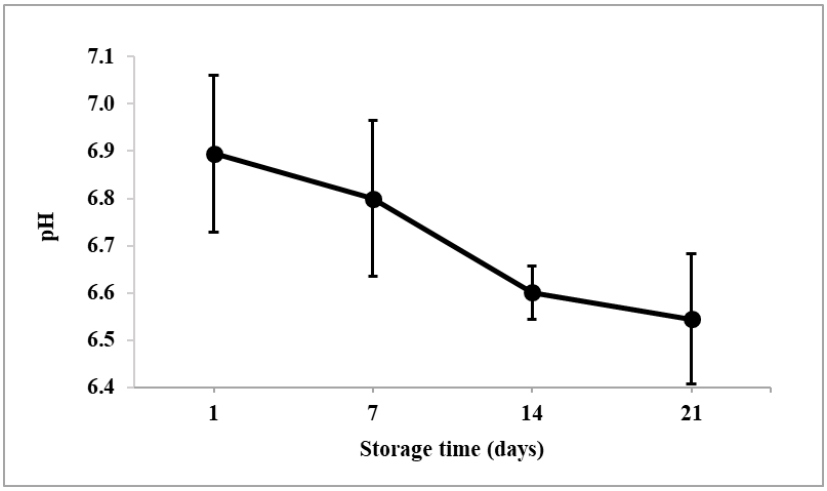

Figure 3. pH of homogenised buffalo Ricotta cheese during storage.

The increase in LAB during product storage could explain the pH decrease (MUCCHETTI *et al.*, 2002). According to HOUGH *et al.* (1999), the pH of Ricotta cheese packaged at temperatures between 65-68°C and stored at 6°C for 20 days decreased to approximately 6.0, and a significant correlation between pH and microbial growth was found. The pH of

fresh traditional sheep Ricotta cheese packaged in a modified atmosphere decreased from 6.54 on day 1 to 5.97 on day 21 of storage (Mancuso *et al.*, 2014).
The redox potential (Fig. 4) ranged from a minimum of 121 mV on day 1 to a maximum of 134 mV on day 14.

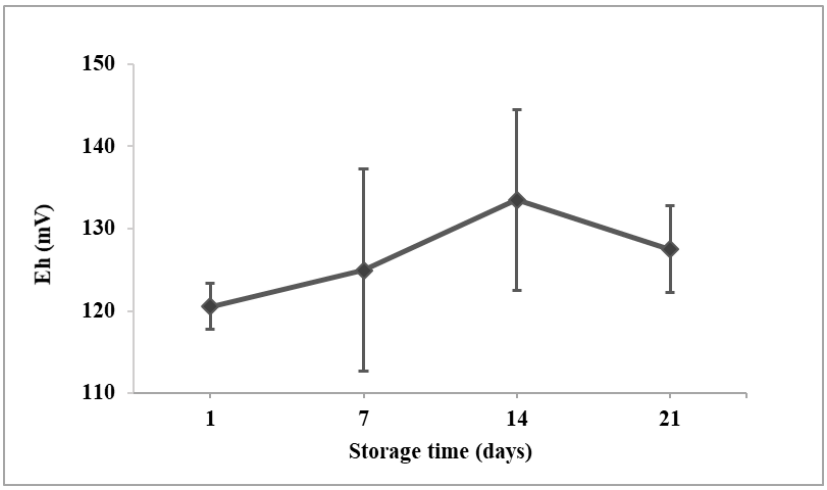

Figure 4. Redox potential of homogenised buffalo Ricotta cheese during storage.

The values found in industrial cow Ricotta cheese were positive (183 mV) (MUCCHETTI *et al.*, 2002). In the samples of homogenised buffalo Ricotta cheese not subjected to freezing before analysis, the redox potential was 82.3 mV (data unpublished).
Redox potential is an important parameter related to food stability. It is affected by many chemical and biological reactions (TANGO and GHALY, 1999), but it is not routinely measured (CALDEO, 2015).
The total antioxidant activity (Fig. 5) measured as Trolox eq. increased slightly from day one (13.69 mmol/100 g or 64.60%) to day 21 (14.05 mmol/100 g or 66.30%).

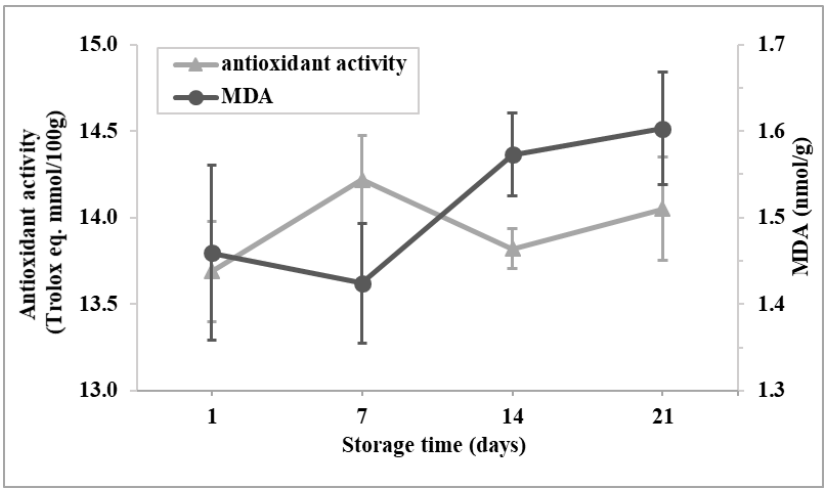

Figure 5. Antioxidant activity and MDA content in homogenised buffalo Ricotta cheese during storage.

In Cheddar cheese, antioxidant activity decreases with an increasing storage period, and in one study was 48% at the beginning of storage and 32% after three weeks of storage (LEE *et al.*, 2016). In another study, the antioxidant activity of Gouda cheese increased throughout the ripening period from 4.61% on day 0 to 16.38% on day 90 (KHAN *et al.*, 2018). The authors of the latter study attributed this increase to water-soluble peptides that have antioxidant properties.

The total antioxidant capacity measures the activity of many antioxidants that are active (KORPELA *et al.*, 1995; LINDMARK-MANSSON and AKESSON, 2000), and variations in activity depend on processing conditions. In milk, the changes upon heating were attributed not only to the thermal degradation of naturally occurring antioxidants, such as vitamins and enzymes, but also to the formation of novel oxidative species (CALLIGARIS *et al.*, 2004; ANDREI *et al.*, 2015).

In Figure 5, the MDA values are shown to slightly increase during storage, with a lower content at day seven (1.42 nmol/g or 0.103%) and a higher content at day 21 (1.60 nmol/g or 0.116%).

In contrast, one study found that the MDA content of buffalo Mozzarella cheese significantly increased after four days of storage (TATICCHI *et al.*, 2017). An increase in MDA content was also detected during the storage of high moisture Mozzarella from cow's milk (SEGAT *et al.*, 2013).

The average MDA content of homogenised Ricotta cheese (1.51 nmol/g or 0.11 mg MDA/kg) was similar to that of Grana Padano cheese (1.28 nmol/g) (Fedele and Bergamo, 2001) and semi-cooked Pecorino cheese (0.13 mg/kg) (BRANCIARI *et al.*, 2014).

In homogenised buffalo Ricotta cheese, a negative correlation (-0.76892; $P \leq 0.015$) between total antioxidant capacity and MDA content was found. Other Authors have confirmed the active role of antioxidants in preventing or limiting fat oxidation. FEDELE and BERGAMO (2001) reported a positive correlation between MDA and consumption of α-tocopherol.

The MDA values of homogenised Ricotta cheese are far below those of other animal and vegetable foods (PAPASTERGIADIS *et al.*, 2012), despite the high fat content of this product. In fact, one of the factors that predisposes to lipid oxidation is the high fat content of food (DALSGAARD *et al.*, 2010; CITTA *et al.*, 2017).

The carbonyl content is shown in Fig. 6 and decreased from 3.93 nmol/mg protein at day one to 3.01 nmol/mg protein at day 21; the average content was 3.52 nmol/mg.

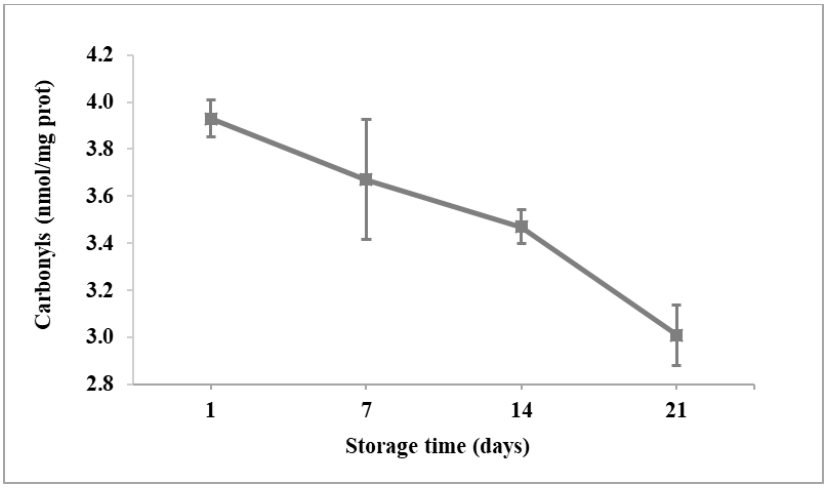

Figure 6. Protein-bound carbonyl content in homogenised buffalo Ricotta cheese during storage.

In comparison with that of other cheeses (FEDELE and BERGAMO, 2001; BALESTRIERI *et al.*, 2002), the carbonyl content of homogenised Ricotta cheese is similar to that of cooked cheeses, such as Grana Padano, Caciocavallo, Provolone and Pecorino Romano, and higher than that of fresh cheeses, including buffalo Mozzarella cheese.
Protein-bound carbonyl groups formed during heat treatment are currently used to evaluate the extent of protein oxidation (FEDYELE and BERGAMO, 2001; FEYNAILLE *et al.*, 2006). Moreover, carbonyl content was positively correlated with the temperature of heat treatment (FEDEYLE and BEYRGAMO, 2001; SCALOYNI *et al.*, 2002).

4. CONCLUSIONS

In conclusion, the process adopted to produce homogenised buffalo Ricotta cheese ensured that during storage, microorganisms considered hygiene markers were below the detection limit. However, the increase in total mesophilic and lactic bacteria during storage suggests stricter control of environmental contaminants. Higher temperatures are also recommended during the second heat treatment to reduce the number of heat-resistant microorganisms and to contain the acidification of the product.
Owing to its oxidative characteristics, homogenised buffalo Ricotta cheese is comparable to semi-cooked and cooked cheeses. The low content of malondialdehyde, which remains almost unchanged at the end of storage, confirms the active role of antioxidants present in dairy products in preventing or limiting fat oxidation.
The suggested process meets the needs of buffalo dairy producers to obtain a product with a longer shelf life and to increase the distribution to national and international markets away from the production area of origin.

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REFERENCES

Addeo F. and Coppola S. 1983. Aspetti tecnologici e microbiologici della trasformazione del latte di bufala in mozzarella e ricotta. Il latte 8:706-723.

Andrei S., Bunea A., Pintea A., Matei S. and Cozma A.M. 2015. Comparative study of antioxidant activity in fresh and pasteurized goat milk. Bulletin UASVM Veterinary Medicine 72:336-340.

Association of Official Analytical Chemists international. 2000. 17th ed. Official Methods of Analysis, Gaithersburg, MD, USA.

Augustyniak E., Adam A., Wojdyla K., Rogowska-Wrzesinska A., Willetts R., Korkmaz A., Atalay M., Weber D., Grune T., Borsa C., Gradinaru D., Bollineni R.C., Fedorova M. and Griffiths H.R. 2015. Validation of protein carbonyl measurement: A multi-centre study. Redox Biology 4:149-157. DOI: doi.org/10.1016/j.redox.2014.12.014.

Balestrieri M., Spagnuolo M.S., Cigliano L., Storti G., Ferrara L., Abrescia P. and Fedele E. 2002. Evaluation of oxidative damage in mozzarella cheese produced from bovine or water buffalo milk. Food chemistry 77(3):293-299. DOI: doi.org/10.1016/S0308-8146(01)00347-8.

Bergamo P., Fedele E., Balestrieri M., Abrescia P. and Ferrara L. 1998. Measurement of malondialdehyde levels in food by high-performance liquid chromatography with fluorometric detection. J. agr. food chem. 46(6):2171-2176.

Branciari R., Ranucci D., Trabalza-Marinucci M., Codini M., Orru M., Ortenzi R., Forte C., Ceccarini M.R. and Valiani A. 2014. Evaluation of antioxidant stability of Pecorino cheese made from raw milk of ewes fed *Rosmarinus officinalis* L. leaves. Intern. J. Food Sci. Tech., 50:558-65.

Caldeo V. 2015. Oxidation-reduction potential and its influence on Cheddar cheese quality. PhD Thesis, University College Cork.

Calligaris S., Manzocco L., Anese M. and Nicoli M.C. 2004. Effect of heat-treatment on the antioxidant and pro-oxidant activity in milk. Int. Dairy j. 14:421-427. DOI: doi.org/10.1016/j.idairyj.2003.10.001.

Casti D., Scarano C., Pala C., Cossu F., Lamon S., Spanu V., Ibba M., Mocci A.M., Tedde F., Nieddu G., Spanu C. and De Santis E.P.L. 2016. Evolution of the microbiological profile of vacuum-packed ricotta salata cheese during shelf-life. Ital. J. Food Safety 5:5501:57-60.

Cherchi A., Porcu M., Spanedda L. and Tuberoso C.I.G. 1999. Caratteristiche chimico-fisiche, organolettiche e microbiologiche di ricotta ovina prodotta in Sardegna. La Rivista di Scienze dell'Alimentazione 28:307-314.

Citta A., Folda A., Scaloni V., Scutari G., Bindoli A., Bellamio M., Feller E. and Rigobello M.P. 2017. Oxidative changes in lipids, proteins, and antioxidants in yogurt during shelf life. Food Sci. Nutr. 5 (6):1079-1087

Dalsgaard T.K., Sørensen J., Bakman M., Vognsen L., Nebel C., Albrechtsen R. and Nielsen J.H. 2010. Light-induced protein and lipid oxidation in cheese: Dependence on fat content and packaging conditions. Dairy Sci. Technol. 90:565-577.

Farkye N.Y. 2004. Acid- and Acid/Rennet-curd Cheeses. Part C: Acid-heat coagulated Cheeses. In: Cheese. Chemistry, Physics and microbiology. Third Edition. Edited by Patrick F. Fox, Paul L.H. McSweeney, Timothy M. Cogan and Timothy P. Guinee pp. 343-348.

Fedele E. and Bergamo P. 2001. Protein and lipid oxidative stresses during cheese manufacture. J. Food Sci. 66(7):932-935. DOI: doi.org/10.1111/j.1365-2621.2001.tb08214.x.

Fenaille F., Parisod V., Visani P., Populaire S., Tabet J.-C. and Guy P. A. 2006. Modifications of milk constituents during processing: A preliminary benchmarking study. Int. Dairy J. 16:728-739. DOI: https://doi.org/10.1016/j.idairyj.2005.08.003.

Feng X., Li C., Ullah N., Cao J., Lan Y., Ge W., Hackman R.M., Li Z. and Chen L. 2015. Susceptibility of whey protein isolate to oxidation and changes in physicochemical, structural, and digestibility characteristics. J. Dairy Sci. 98(11):7602-7613. DOI: https://doi.org/10.3168/jds.2015-9814.

FIL-IDF 172: 2001 / ISO 14156:2001- Milk and Milk products-extraction methods for lipids and liposoluble compounds.

FIL-IDF. 1991. Chemical methods for evaluating proteolysis in cheese maturation, No. 261. Brussels, Belgium: International Dairy Federation.

FIL-IDF. 1993. Milk. Determination of nitrogen content. Kjeldahl method; Standard 20B. Annex I: Modified procedure for milk products. Brussels; Belgium: International Dairy Federation.

Hough G., Puglieso M.L., Sanchez R. and Da Silva O.M. 1999. Sensory and microbiological shelf-life of a commercial Ricotta cheese. J. Dairy Sci. 82(3):454-459. DOI: doi.org/10.3168/jds.S0022-0302(99)75253-7.

International Dairy Federation. 1986. Cheese and processed cheese products. Determination of dry matter. IDF Standard No. 4. FIL-IDF, Brussels, Belgium.

International Dairy Federation. 1988. Determination of dry salt content. IDF Standard No. 12B. FIL-IDF, Brussels, Belgium.

Katalinic V., Milos M., Kulisic T. and Jukic M. 2006. Screening of 70 medicinal plant extracts for antioxidant capacity and total phenols. Food Chemistry 94:550-557. DOI: doi.org/10.1016/j.foodchem.2004.12.004.

Khan I.T., Nadeem M., Imram M., Ajmal M. and Ali S. 2018. Antioxidant activity, fatty acids characterization and oxidative stability of Gouda cheese fortified with mango (*Mangifera indica* L.) kernel fat. J. Food Sci. Technol. 155:992-1002. DOI: doi.org/10.1007/s13197-017-3012-y.

Korpela R., Ahotupa M., Korhonen H. and Syvaoja E.L. 1995. Antioxidant properties of cow's milk. In: Proceedings of the NJF/NMR, Seminar n. 252, pp. 157-159, Turku, Finland.

Lee N.K., Jeewanthi R.K.C., Park E.H. and Paik H.D. 2016. Short communication: Physicochemical and antioxidant properties of Cheddar-type cheese fortified with *Inula Britannica* extract. J. Dairy Sci. 99:83-88. DOI: doi.org/10.3168/jds.2015-9935.

Lindmark-Mansson H. and Akesson B. 2000. Antioxidant factors in milk. British Journal of Nutrition, 84 (1):103-110. DOI: doi.org/10.1017/S0007114500002324.

Lipan L., Sánchez-Rodríguez L., Cano-Lamadrid M., Collado-González J., Noguera-Artiaga L., Sendra E. and Carbonell-Barrachina A.A. 2017. Modernization of traditional food processes and products. In: Consumer trends and new product opportunities in the food sector. Edited by Klaus G. Grunert. pp. 113-133. DOI: doi.org/10.3920/978-90-8686-852-0_8

Mancuso I., Cardamone C., Fiorenza G., Macaluso G., Arcuri L., Miraglia V. and Scatassa M.L. 2014. Sensory and microbiological evaluation of traditional ovine ricotta cheese in modified atmosphere packaging. Ital. J. Food Safety 3(2):1725-1727. DOI: doi.org/10.4081/ijfs.2014.1725.

Maubois J.L. and Kosikowski F.V. 1978. Making Ricotta cheese by ultrafiltration principles. J. Dairy Sci. 61:881-884.

Modler H.W. and Emmons D.B. 1989. Production and yield of whole milk Ricotta manufactured by a continuous process. II. Results and discussion. Milchwissenschaft 44:673-676.

Mortensen G., Bertelsen G., Mortensen B.K. and Stapelfeldt H. 2004. Light-induced changes in packaged cheeses - a review. Int. Dairy J. 14:85102. DOI: doi.org/10.1016/S0958-6946(03)00169-9.

Mucchetti G., Carminati D. and Pirisi A. 2002. Ricotta fresca vaccina ed ovina: osservazioni sulle tecniche di produzione e sul prodotto. Latte 27:154-166.

Mucchetti G. and Neviani E. 2006. Microbiologia e tecnologia lattiero-casearia. Tecniche Nuove (ed), Milano, IT.

Mucchetti G., Pugliese A. and Paciulli M. 2017. Characteristics of some important Itaian cheeses - Parmigiano Reggiano, Grana Padano, Mozzarella, Mascarpone and Ricotta. In: Cruz RMS, Veira MC (eds) Mediterranean foods. CRT Press, London.

Nikolova M., Evstatieva L. and Nguyen T.D. 2011. Screening of plant extracts for antioxidant properties. Botanica Serbica 35 (1):43-48.

Ottogalli G., Rondinini G. and Cappelletti C. 1981. Ricerche su alcuni aspetti microbiologici ed igienici delle ricotte. Annali di Microbiologia ed Enzimologia 31:77-87.

Palmas F., Carta A., Cosentino S., Fadda M.E., Gilberto G. and Mulargia A.F. 1994. Nuova tecnologia per la produzione di ricotta ovina: caratteristiche e valutazione della conservabilità. La Rivista di Scienze dell'Alimentazione 23:467-472.

Papastergiadis A., Mubiru E., Van Langenhove H. and De Meulenaer B. 2012. Malondialdehyde measurement in oxidized food: evaluation of the spectrophotometric thiobarbituric acid reactive substance (TBARS) test in various food. J. Agric. Food Chem. 60:9589-9594. DOI: doi.org/10.1021/jf302451c.

Pintado M.E and Malcata F.X. 2000. Characterization of whey cheese packaged under vacuum. J. Food Protection 63(2):216-221. DOI: doi.org/10.4315/0362-028X-63.2.216.

Pintado M.E., Macedo A.C. and Malcata F.X. 2001. Review: technology, chemistry and microbiology of whey cheeses. Food Sci. Technology Intern. 7:105-116. DOI: doi.org/10.1177/108201320100700202.

Raharjo S. and Sofos J. N. 1993. Methodology for measuring malonaldehyde as a product of lipid peroxidation in muscle tissues: A review. Meat Science 35(2):145-169. DOI: doi.org/10.1016/0309-1740(93)90046-K.

Raia G., Fedele E., Balestrieri M. and Bergamo P. 1996. Specific and sensitive HPLC method for malondialdehyde measurement in milk and cheese. In Food and Health: role of animal products. pp. 205-209 (Enne G. and Greppi G.F. eds.) Elsevier press, Amsterdam.

Reznick A.Z. and Packer L. 1994. Oxidative damage to proteins: spectrophotometric method for carbonyl assay. In Methods in enzymology (Vol. 233, pp. 357-363) Academic Press.

Salvadori Del Prato O. 2001. Trattato di Tecnologia Casearia. Calderini Edagricole, Bologna, IT.

Salvatore E., Pes M., Falchi G., Pagnozzi D., Furesi S., Fiori M., Addis M.F. and Pirisi A. 2014. Effect of whey concentration on protein recovery in fresh ovine ricotta cheese. J. Dairy Sci. 97:4686-4694. DOI: doi.org/10.3168/jds.2013-7762.

Scaloni A., Perillo V., Franco P., Fedele E., Froio R., Ferrara L. and Bergamo P. 2002. Characterization of heat-induced lactosylation products in caseins by immunoenzymatic and mass spectrometric methodologies. Biochim. Biophys. Acta 1598:30-39.

Scarano C., Spanu C., Mocci A.M., Piras F., Demontis M., Murittu G., Pinna G., Santoru A., and De Santis E.P.L. 2019. Microbiological and physicochemical properties of smoked ricotta cheese during refrigeration and temperature abuse storage. Ital. J. Food Safety 8:8009, 102-109. DOI: doi.org/10.4081/ijfs.2019.8009

Segat A., Biasutti M., Iacumin L., Comi G., Baruzzi F., Carboni C. and Innocente N. 2013. Use of ozone in production chain of high moisture Mozzarella cheese. Food Sci. Tech. 55:513-520. DOI: doi.org/10.1016/j.lwt.2013.10.029.

Shahani K.M. 1979. Newer techniques for making and utilization of Ricotta cheese. Proc. 1st Biennal Marschall International Cheese Conference, Madison, WI, 77-87.

Smithers G.W. 2008. Whey and whey protein – From "gutter-to-gold". Int. Dairy J. 18(7):695-704. DOI: doi.org/10.1016/j.idairyj.2008.03.008.

Tango M.S.A. and Ghaly A.E. 1999. Amelioration of lactic acid production from cheese whey using micro-aeration. Biomass and Bioenergy17:221-238. DOI: doi.org/10.1016/S0961-9534(99)00037-9.

Taticchi A., Bartocci S., Servili M., Di Giovanni S., Pauselli M., Mourvaki E., Meo Zilio D. and Terramoccia S. 2017. Effect on quanti-quality milk and mozzarella cheese characteristics with further increasing the level of dried stoned olive pomace in diet for lactating buffalo. Asian-Australas. J. Anim Sci. 30:1605-1611. DOI: doi.org/10.5713/ajas.16.0767.

True L.C. 1973. Effect of various processing conditions on the yield of whey Ricotta cheese. Proc. 10th Marschall Invitational Cheese Seminar, Madison, WI, 1-11.

Unal G. 2012. Antioxidant activity of commercial dairy products. AgroFOOD Industry hi-tech 23(1):39-42.

Weatherup W. 1986. The effect of processing variables on the yield and quality of Ricotta. Dairy Ind. Int. 5:41-45.

Wilbey R.A., Scott J.E., Robinson R.K. 2012. Cheesemaking practice. Springer Science & Business Media. DOI: doi.org/10.1007/978-1-4615-5819-4.

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