Microbiological and physicochemical properties of smoked ricotta cheese during refrigeration and temperature abuse storage

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Abstract

In the last years changes occurred in the production process of *ricotta mustia*, a traditional smoked, salted and sometimes ripened ricotta cheese, produced in Sardinia. Fresher, slightly smoked and with reduced salt content products, were introduced into the market to meet changes in consumer's preferences for milder products. The present study of durability was conducted on an innovative fresh and smoked industrial product, also characterized by the small size and the packaging in modified atmosphere. A durability test to assess the evolution of microbiological and physicochemical profile of the product stored at refrigeration (4°C) and mild abuse (7°C) temperatures was carried out. A total of 126 ricotta samples smoked for either 1, 2, or 3 h were analyzed at intervals during shelf-life for the determination of aerobic mesophilic counts, *Enterobacteriaceae*, yeast, moulds, *L. monocytogenes*, *Pseudomonas* spp. and *B. cereus*. Intrinsic properties, physic-chemical and headspace gas composition were also analyzed. Average and standard deviation were respectively 6,06±0,22 for pH, 0,982±0,05 for a_W, 74,67%±1,81% for moisture, 10,25%±1,35% for fat, 10,92%±0,46% for protein and 1,70%±0,42% for salt content. Total bacterial count ranged between 3.88±0.48 log cfu/g at T_0 and 3.25±1.02 at T_{45}. *L. monocytogenes*, *Pseudomonas* spp. and *E. coli* were always below the detection limit. *Enterobacteriaceae* prevalence (percentage) was 3.17% (2.62±0.42 lg10 cfu/g) and was limited to samples stored longer than 30 days while *B. cereus* was recovered in 5.55% (2.36±0.35 lg10 cfu/g) of the samples and was never observed in samples after 45 days of refrigerated storage. The durability study is preliminary to challenge test to assess the shelf-life of this product in compliance with the requirements of Regulation (EC) 2073/2005.

Introduction

Whey is the fluid product obtained during the manufacture of cheese, casein or similar products by separation from the curd after coagulation of milk and/or of products obtained from milk (CODEX STAN 289-1995). The main food use of whey is the preparation of whey cheeses, whey drinks and fermented whey drinks (FAO, 2018). Ricotta is a general term used to identify a variety of Italian whey cheese. The manufacturing of ricotta cheese is based on the heat-denaturation of whey proteins up to cause their coagulation. Especially in the Mediterranean basin, there are several traditional ovine whey cheeses manufactured after the production of sheep milk cheeses such as Mizithra, Anthotyros and Manouri (Greece), Anari (Cyprus), Requesón (Spain), Requeijao (Portugal), Brocciu (France), Urdă (Balkans region) (Casti *et al.*, 2016). Sardinia is the Italian region leader for dairy sheep industry (Storelli *et al*., 2012). Typical production from sheep milk whey consist of fresh (*ricotta fresca*), salted (*ricotta toscanella*, *ricotta testa di morto* and *ricotta moliterna*) or salted, smoked and sometimes ripened (*ricotta mustia*) ricotta cheese. These products are included in the "List of Traditional Agri-Food Products" of the Italian Ministry of Agricultural, Food and Forestry Policies (Ministerial Decree 18 luglio 2000). Smoked ricotta cheese is manufactured in artisanal (*Ricotta mustia)* or industrial cheesemaking plants (*ricotta toscanella)* by pressing the curd to enhance drainage, then dry salted and smoked, at artisanal level by combustion of aromatic woods in a fireplace while at industrial level into a smoking chamber. Changes in consumer's lifestyle and preferences has driven food business operators to place new ricotta cheese product on the market, competing with local foods. In Sardinia, the ricotta production process has already been implemented, both at industrial and at artisanal level, including innovation steps such as MAP packaging, use of protective cultures to control secondary contaminations (Pala *et al.,* 2016; Spanu *et al.,* 2017, 2018) or the use of lactase to reduce the lactose content (Pulinas *et al.,* 2017) in *ricotta fresca*, post-lethality thermal treatment in *ricotta salata* (Spanu *et al*., 2013, 2015a,b). A possible strategy of product innovation is the modernization of traditional food processes and products (McElhatton and El Idrissi, 2016). Innovation of traditional foods can be obtained by changing the manufacturing process through the introduction of production steps, changes in product composition, packaging, product size and form or new ways to use the product (Gellynck and Kühne, 2008; Lipan *et al.,* 2017). Following these trends, in recent years, has been observed a rise in the demand of small sized or single serving packaged ricotta cheese (Troiani, 2015). Some changes occurred also in the traditional smoked *ricotta salata* cheese (*ricotta mustia*) produced in Sardinia, with a fresher, slightly smoked and with reduced salt content products, introduced into the market to meet changes in consumer's preferences. With the purpose of meeting consumer's demand for fresh and healthy dairy products combined with the sensory attribute of the traditional product, an industrial sheep cheese making plant located in Sardinia (Italy) developed a product innovation of the traditional *ricotta mustia*. The innovated product, a fresh small sized, slightly salted ricotta cheese in MAP packaging, was developed to meet the GDO demand. In further sections of this paper, it will be referred as *novel smoked ricotta cheese (NS-Ricotta Cheese)*. However, any time major process or formulation changes occurs, prior to place the food on the market, food business operator who aim to produce safe, wholesome and attractive food products, should conduct a proper shelf-life evaluation (Man and Jones, 1994).

The objective of the present study was to evaluate the impact of smoking time and

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temperature of storage on the microbiological profile and physicochemical properties of smoked ricotta cheese during shelf life. With this aim a durability study was conducted on MAP packed NS-Ricotta samples stored at refrigeration (4°C) and mild abuse (7°C) temperature for up to 45 days.

Materials and Methods

NS-Ricotta Cheese samples

The study was conducted in collaboration with an industrial sheep's milk cheese making plant located in Sardinia (Italy) which has developed and produced the NS-Ricotta Cheese samples used in the present experiment. Three NS-Ricotta Cheese batches were manufactured in different production days according to the following production steps. The whey remaining after the daily cheese production was filtered to remove curd residues and stored in a stainless silo tank until use. The whey was cleaned by centrifugal separators before being preheated to 60-70°C passing through a plate heat exchanger and then transferred into open kettles of 1,200-1500 l of capacity. The whey was heated using a direct steam injection system until the temperature reached ca. 80°C. The foam produced during heating was removed from the whey surface. As the flocculated proteins started to rise, heating was interrupted and the mixture held in the vat for ca. five minutes. Clots were then collected with the use of perforated ladles and transferred into plastic basket placed on drainage tables. Ricotta basket were allowed to drain and to cool for about ten-fifteen minutes after which the inner temperature of the curd dropped from 72-75°C to ca. 65-70°C. Ricotta basket was transferred in a cold room at 4°C for 18-20 hours. After refrigeration, the ricotta cheese was dry salted and cold smoked (25-30°C) in a smoking chamber for either one, two or three hours. The smoke was produced by burning of beech (*Fagus sylvatica*) shavings. After 24 h of refrigerated storage (4°C) in cold room, smoked ricotta cheese was packed in modified atmosphere using rigid polypropylene trays sealed with high-barrier peelable laminated films. The gas mixture used was 90% N and 10% CO_2. The final product was a truncated cone shaped cheese with upper base ca. 6.5 cm wide, lower base ca. 8.5 cm wide, height of ca. 6 cm and weigh of approximately 300 g. The main differences in the manufacturing process of the *NS-Ricotta Cheese* as compared to *ricotta mustia* or *toscanella* are the absence of the pressing step, the cold smoking instead of hot smoking, the smaller size and weight (300 g *vs*. 800-2,000 g) and MAP packaging instead of food wrapping paper. The flowchart of the *NS-Ricotta Cheese* making process is represented in Figure 1. After packaging samples were stored refrigerated until analysis were performed.

Experimental design

A total of 126 NS-Ricotta Cheese samples were used, 42 from each of three production batches. From each batch fourteen ricotta samples were smoked for each of the following smoking time: 1, 2 and 3 hours (H1, H2 and H3). The durability study was conducted analyzing NS-Ricotta Cheese samples at different moment during the shelf-life. Sampling times were: within 24 h after packaging, defined as time zero (T0), time 15 (T15), time 30 (T30) and time 45 (T45), respectively 15 days, 30 days and 45 days after packaging. In order to account for temperature abuse during the storage period, for each batch, for each smoking time and at each time point, duplicate ricotta sample were stored both at 4°C and at 7°C. At T0 samples were kept only at 4°C with no thermal abuse. Table 1 reports the number of samples and the analysis performed at each sampling time.

Microbiological analysis

The preparation of the initial suspension and decimal dilution for microbiological examination was conducted according to ISO 6886-1:1999. From each sample two 25 g aliquots were aseptically collected and homogenized, one with 225 mL of Fraser Broth Base FBB (Biolife, Milan, Italy) for the detection of *L. monocytogenes* and one with 225 mL of Buffered Pepton Water BPW (Biolife) for all other parameters. After homogenization, serial decimal dilutions were prepared in BPW solution and used to

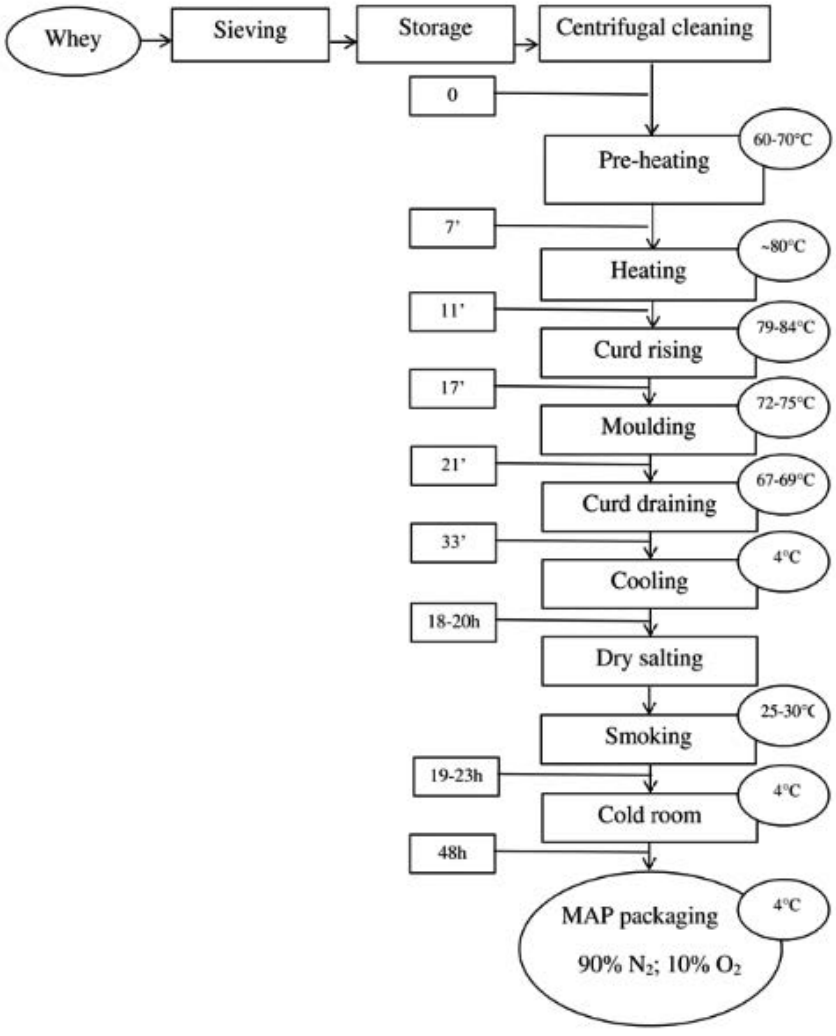

Figure 1. Flowchart of the NS-Ricotta Cheese making process.

inoculate the appropriate culture media. The pour-plating procedure was used for the enumeration of aerobic mesophilic counts (ISO 4833; ISO, 2003), *Enterobacteriaceae* (ISO 21528-2; ISO, 2004), yeast and moulds (ISO 6611/IDF 94, 2004). The spread plating technique was used for the enumeration of *L. monocytogenes* (ISO 11290-1/2; ISO 1996, 1998), *Pseudomonas spp* (ISO/TS 11059:2009; ISO, 2009) and *B. cereus* (ISO 7932; ISO 2004).

Physicochemical analysis and composition

Intrinsic properties such as PH and a_w were measured using pH meter GLP22 (Crison Instruments, Barcelona, Spain) and water activity meter Aqualab 4TE (Decagon, Pullman, WA, USA), respectively. Fat, moisture, protein and total solids were analyzed by using the compositional FoodScan™ device (FOSS, Analytic, Hillerød, Denmark), which uses the near-infrared spectrophotometer system.

Headspace gas composition

The determination of the headspaces gas composition was conducted on sealed NS-Ricotta Cheese samples before performing other analysis. Measures were obtained piercing the lid using a sterile needle connected to the Dansensor gas analyser (PBI Dansensor, Ringsted, Denmark). To avoid gas leaks during the penetration of needle, 15 Ø mm septum (PBI Dansensor), were applied on the film lid before measurements of headspace gas composition. Measures of combined residual O_2% and CO_2% were directly read on the instrument while N_2 was calculated by difference.

Statistical analysis

Differences among mean microbiological counts (cfu g^{-1}), headspace gas concentration (%), intrinsic properties (pH and a_W) and centesimal composition (%) over time (T_0, T_{15}, T_{30} and T_{45}) were compared using Fisher's least significant difference (LSD) test using Statgraphics Centurion XVI software (Stat Point Technologies, Warrenton, VA, USA). To account for the effect of smoking time (three levels: 1, 2 and 3 hours), storage temperature (two levels: 4°C and 7°C) and storage duration (4 levels:

Table 1. Number of *NS-Ricotta Cheese* samples and analysis performed at each day of storage by length of smoking.

Parameters	Smoking[4]	Day of storage						
		T_0	T_{15}		T_{30}		T_{45}	
		4°C	4°C	7°C	4°C	7°C	4°C	7°C
Microbiological profile[1], Physico-chemica[l2] and headspace gas[3] composition	H_1	6	6	6	6	6	6	6
	H_2	6	6	6	6	6	6	6
	H_3	6	6	6	6	6	6	6

[1]Total aerobic mesophilic counts, *Enterobacteriaceae*, *E. coli*, *L. monocytogenes*, *B. cereus*, *Pseudomonas* spp, yeast and moulds; [2]pH, aW, moisture%, fat%, protein% and salt%; [3]O_2%, CO_2% and N_2%; [4]smoking time hour (H): 1, 2 and 3.

Table 2. Evolution of the microbiological profile (log_{10} cfu/g; $\bar{x}$ ±SD) of MAP *NS-Ricotta Cheese* by smoking time during storage at 4°C.

Microbial group	Smoking	Day of storage			
		T_0	T_{15}	T_{30}	T_{45}
Aerobic mesophilic bacteria	H_1	(n = 6/6) 4.05±0.23^{a1}	(n = 6/6) 2.82±0.36^{c1}	(n = 6/6) 3.15±0.83^{bc1}	(n = 6/6) 3.78±1.09^{ab1}
	H_2	(n = 6/6) 3.56±0.74^{a1}	(n = 6/6) 2.76±0.51^{ab1}	(n = 6/6) 2.69±0.96^{ab1}	(n = 6/6) 2.68±0.61^{b1}
	H_3	(n = 6/6) 4.04±0.15^{a1}	(n = 6/6) 2.32±0.32^{b1}	(n = 6/6) 2.76±0.74^{b1}	(n = 6/6) 2.88±0.51^{b1}
Enterobacteriaceae	H_1	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 1/6) 2.77±0.0
	H_2	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.
	H_3	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.
E. coli	H_1	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.
	H_2	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.
	H_3	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.
Yeast	H_1	(n = 0/6) N.D.	(n = 1/6) 1.30±0.0^{a}	(n = 3/6) 2.78±0.68^{a}	(n = 1/6) 3.08±0.0^{a}
	H_2	(n = 0/6) N.D.	(n = 1/6) 1.60±0.0^{a}	(n = 1/6) 2.00±0.0^{a}	(n = 0/6) N.D.
	H_3	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 1/6) 3.08±0.0^{a}	(n = 2/6) 3.04±0.19^{a}
Molds	H_1	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 3/6) 2.26±0.24^{a}	(n = 1/6) 3.00±0.0^{a}
	H_2	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 1/6) 2.48±0.0^{a}	(n = 0/6) N.D.
	H_3	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 2/6) 2.65±0.49^{a}	(n = 1/6) 2.47±0.0^{a}
Pseudomonas spp	H_1	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.
	H_2	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.
	H_3	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.
Bacillus cereus	H_1	(n = 0/6) N.D.	(n = 1/6) 2.84±0.0^{a}	(n = 1/6) 2.00±0.0^{a}	(n = 0/6) N.D.
	H_2	(n = 1/6) 2.60±0.0^{a}	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.
	H_3	(n = 0/6) N.D.	(n = 1/6) 2.00±0.0^{a}	(n = 0/6) N.D.	(n = 0/6) N.D.
Listeria monocytogenes	H_1	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.
	H_2	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.
	H_3	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.

T_0= day of packaging; T_{15}, T_{30} and T_{45} = respectively, 15, 30 and 45 days of storage; H_1, H_2 and H_3 indicate the smoking time respectively of 1, 2 and 3 hours; means in the same row with different superscript letter indicate a significant difference (P<0.05) among days of storage; for each microbial group means in the same column with different superscript number indicate a significant difference (P<0.05) among smoking time. Values within brackets indicate the prevalence of positive samples.

0, 15, 30 and 45 days) on physico-chemical and headspace gas composition, a General Linear Model was conducted using the Multifactor ANOVA procedure of Statgraphics Centurion XVI software (Stat Point Technologies).

Results

Microbiological profile

Total bacterial count ranged between 3.88±0.48 log_{10} cfu/g at T_0 and 3.24±1.02 log_{10} cfu/g at T_{45}. The bacterial count was significantly affected by smoking time (P<0.001; H_2 showing values 0.49 log_{10} lower than H_1) and days of storage (P<0.001; a decrease of 1.22 log_{10} was observed between T_0 and T_{15}) while the temperature of storage showed no effect (P>0.05). Yeast and molds were occasionally reported in the first fifteen days of storage, respectively in 2.6% (1.45±0.21 log_{10} cfu/g) and in 1.3% (2.00±0.0 log_{10} cfu/g) of the samples. During the storage, the prevalence increased to 29.1% for yeast (2.88±0.59 log_{10} cfu/g) and to 22.2% for molds (2.36±0.37 log_{10} cfu/g). *B. cereus* was observed in four samples (5.6%) stored at 4°C and in three samples (4.1%) stored at 7°C; mean count was 2.36±0.35 log_{10} cfu/g. *Enterobacteriaceae* were enumerated in one samples stored at 4°C for forty-five days (2.77 log_{10} cfu/g) and in three samples stored at 7°C of which one for thirty days (2.00 log_{10} cfu/g) and two for forty-five days (2.87±0.04 log_{10} cfu/g). *E. coli*, *Pseudomonas* spp and *Listeria monocytogenes* were never detected. Tables 2 and 3 report the complete microbiological profile with mean counts (log10 cfu/g; ±SD) over time by smoking time, respectively for *NS-Ricotta Cheese* stored at 4°C and 7°C.

Physicochemical and headspace gas composition

The pH of ricotta salata cheese during refrigerated storage under vacuum packing decreased from an initial level (T_0) of 6.31±0.04 to a final level (T_{45}) of 5.96±0.18 (P<0.05); smoking time and days of storage showed a significant effect (P<0.05) while temperature of storage showed no impact on the pH (P>0.05). The a_W values ranged between 0.984±0.005 at T_0 and 0.983±0.005 at T_{45}; no significant difference was observed for smoking time and temperature of storage (P>0.05) while a significant effect of days of storage was observed, with higher values at T_0 and T_{45} (P<0.05). Moisture ranged between 74.55±1.83% at T_0 and 74.65±1.81% at T_{45}, fat ranged between 11.13±1.43 at T_0 and 9.88±1.16 at T_{45}, proteins ranged between 10.36±0.53 at T_0 and 11.14±0.32 at T_{45}, salt content ranged between 1.87±0.34 at T_0 and 1.59±0.45 at T_{45}, respectively. Moisture was significantly affected by smoking time (P<0.001) with lower values observed in ricotta samples smoked for three hours, fat and proteins content were significantly affected (P<0.001) by days of storage while the salt content was significantly affected by smoking time (P<0.05) and days of storage (P<0.001). Smoking time and days of storage had a significant impact (P<0.001) on N, CO_2 and O_2% headspace content while temperature of storage had a significant effect (P<0.05) on the O_2% content (Tables 4 and 5).

Discussion

In recent years, the global interest for local foods has increased due to their perceived greater quality as compared to conventional foods (Aprile *et al.,* 2016). In particular, traditional food products constitute an important element of European culture, identity and heritage (Almli *et al.,* 2011; Vanhonacker *et al*., 2010). Consumer's attitude toward traditional foods is positive

Table 3. Evolution of the microbiological profile (log_{10} cfu/g; $\bar{x}$ ±SD) of MAP *NS-Ricotta Cheese* by smoking time during storage at 7°C.

Microbial group	Smoking	Day of storage			
		T_0	T_{15}	T_{30}	T_{45}
Aerobic mesophilic bacteria	H_1	(n = 6/6) 4.04±0.23^{a1}	(n = 6/6) 2.79±0.31^{b1}	(n = 6/6) 3.62±1.05^{ab1}	(n = 6/6) 3.48±0.82^{ab1}
	H_2	(n = 6/6) 3.56±0.75^{a1}	(n = 6/6) 2.70±0.61^{a1}	(n = 6/6) 2.75±0.83^{a1}	(n = 6/6) 3.04±1.23^{a1}
	H_3	(n = 6/6) 4.03±0.15^{a1}	(n = 6/6) 2.57±0.54^{b1}	(n = 6/6) 2.94±0.82^{b1}	(n = 6/6) 3.63±1.46^{ab1}
Enterobacteriaceae	H_1	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 1/6) 2.00±0.0	(n = 2/6) 2.87±0.04
	H_2	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.
	H_3	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.
E. coli	H_1	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.
	H_2	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.
	H_3	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.
Yeast	H_1	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 3/6) 2.73±0.60^{a}	(n = 3/6) 2.89±0.11^{a}
	H_2	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 1/6) 3.17±0.00^{a}	(n = 0/6) N.D.
	H_3	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 2/6) 2.60±0.43^{a}	(n = 4/6) 3.16±1.09^{a}
Molds	H_1	(n = 0/6) N.D.	(n = 1/6) 2.00±0.0a	(n = 3/6) 2.10±0.17^{a}	(n = 2/6) 2.30±0.43^{a}
	H_2	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 1/6) 2.00±0.00^{a}	(n = 0/6) N.D.
	H_3	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 2/6) 2.45±0.64^{a}	(n = 0/6) N.D.
Pseudomonas spp	H_1	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 1/6) 3.36±0.0
	H_2	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.
	H_3	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.
Bacillus cereus	H_1	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.
	H_2	(n = 1/6) 2.60±0.00^{a}	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.
	H_3	(n = 0/6) N.D.	(n = 1/6) 2.00±0.00^{a}	(n = 1/6) 2.47±0.00^{a}	(n = 0/6) N.D.
Listeria monocytogenes	H_1	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.
	H_2	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.
	H_3	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.	(n = 0/6) N.D.

T_0= day of packaging; T_{15}, T_{30} and T_{45} = respectively, 15, 30 and 45 days of storage; H_1, H_2 and H_3 indicate the smoking time respectively of 1, 2 and 3 hours; means in the same row with different superscript letter indicate a significant difference (P<0.05) among days of storage; for each microbial group means in the same column with different superscript number indicate a significant difference (P<0.05) among smoking time. Values within brackets indicate the prevalence of positive samples.

since these goods are generally linked to regional identity and sensory quality (Guerrero *et al*., 2009). Introducing innovations into traditional food products seems somehow controversial since it has to improved production process on one hand and preserve the sensory quality seek by consumers on the other (Jordana, 2000). However, in order to maintain and expand the market of traditional food products food industries are now pursuing the further improvement in safety, health and convenience of such products by means of different innovations (Kühne *et al.,* 2010). Food product packaging plays a key role in the innovation of traditional food products, contributing to capture potential consumer's attention and encouraging product purchase (Bloch, 1995; Silayoi and Speece, 2007; Piqueras-Fiszman and Spence, 2012). With regard to packaging, the main innovations introduced in the present study were the use of MAP packaging and the reduction of product size. The absence of the pressing step and the cold smoking instead of hot smoking were the main technological innovations in *NS-Ricotta Cheese* with respect to other traditional salted ricotta cheese. As consequence of the changes in the production process the *novel smoked ricotta cheese* has intrinsic properties (pH 6.2-6.3; a_W 0.984-0.986) that are between the *ricotta fresca* (pH 6.5-6.8; a_W 0.990-0.994) and *ricotta salata* (pH 6.3-6.5; a_W 0.950-0.980). The evolution during storage is similar to what has been observed in ricotta salata with a 0.4-0.5 pH decrease (Casti *et al*., 2016) while a_W, similarly to both *ricotta fresca* and *ricotta salata* remained stable or with little variation (Pala *et al*., 2016; Spanu *et al*., 2013). Food safety criteria (*L. monocytogenes*) and process hygiene criteria (*E. coli*) were always compliant with Regulation (EC) No 2073/2005 (European Commission, 2005). *Enterobacteriaceae* were enumerated only in one sample (1.4%) after 45 days of storage at 4°C and in three samples (4.2%) after 30 days at 7°C, with mean levels always below 3 $\log_{10}$ cfu/g. These values are lower as compared to *ricotta fresca* and *ricotta salata* where mean counts of ca. 6-7 $\log_{10}$ cfu/g and of ca. 4-5 log10 cfu/g were observed respectively after 21 and 60 days of refrigerated storage (Casti *et al*., 2016; Pala *et al.,* 2016). Despite no microbiological criteria has been defined for *Enterobacteriaceae* in whey cheeses that undergone heat treatment, these are important indicator microorganisms, revealing good hygienic conditions during the manufacturing process. *B. cereus* was reported occasionally, with counts of ca. 2 $\log_{10}$ cfu/g, dose below the five to eight $\log_{10}$ cfu/g, generally considered necessary to cause illness (ICMSF, 1996). *B. cereus* vegetative cells were not recovered from samples stored up to 45 days at both 4°C and 7°C. This finding is in agreement with previous investigation conducted on salted ricotta samples, where vegetative cells decreased during refrigerated storage, indicating that the contamination was likely due to mesophilic strains which minimum growth temperature is 15°C (Spanu *et al*., 2016). *Pseudomonas* spp. were never detectable, despite fresh ricotta cheese is particularly susceptible of secondary contamination and represents an excellent substrate for the growth of psychotropic spoilage microorganisms (Ibba *et al*., 2013; Scarano *et al.,* 2014; Spanu *et a*l., 2015c). The present finding is in contrast with previous investigation where *Pseudomonas*

Table 4. pH, a_W, physico-chemical and headspace gas composition ($\bar{x}$ ±SD) of ricotta samples by smoking time (1, 2 and 3 hours) stored at 4°C.

Parameters	Smoking	Day of storage			
		T_0	T_{15}	T_{30}	T_{45}
pH	H_1	(n = 6) 6.32±0.03^{a1}	(n = 6) 6.26±0.15^{ab1}	(n = 6) 6.15±0.07^{b1}	(n = 6) 6.18±0.68^{b1}
	H_2	(n = 6) 6.29±0.03^{a1}	(n = 6) 6.13±0.19^{b1}	(n = 6) 5.94±0.87^{c2}	(n = 6) 5.96±0.11^{c2}
	H_3	(n = 6) 6.32±0.06^{a1}	(n = 6) 6.05±0.27^{b1}	(n = 6) 5.81±1.33^{c3}	(n = 6) 5.80±0.13^{c3}
a_w	H_1	(n = 6) 0.984±0.004^{a1}	(n = 6) 0.981±0.005^{a1}	(n = 6) 0.980±0.004^{a1}	(n = 6) 0.982±0.004^{a1}
	H_2	(n = 6) 0.984±0.005^{a1}	(n = 6) 0.980±0.006^{a1}	(n = 6) 0.979±0.005^{a1}	(n = 6) 0.982±0.005^{a1}
	H_3	(n = 6) 0.986±0.005^{a1}	(n = 6) 0.979±0.006^{b1}	(n = 6) 0.980±0.003^{b1}	(n = 6) 0.983±0.004^{ab1}
Moisture (%)	H_1	(n = 6) 75.08±1.17^{a1}	(n = 6) 75.12±2.61^{a1}	(n = 6) 74.91±0.60^{a1}	(n = 6) 75.00±1.63^{a1}
	H_2	(n = 6) 74.92±2.29^{a1}	(n = 6) 74.92±2.03^{a1}	(n = 6) 74.78±1.73^{a1}	(n = 6) 75.48±1.91^{a1}
	H_3	(n = 6) 73.66±1.92^{a1}	(n = 6) 73.92±1.46^{a1}	(n = 6) 74.47±1.22^{a1}	(n = 6) 73.95±2.05^{a1}
Fat (%)	H_1	(n = 6) 10.89±1.01^{a1}	(n = 6) 10.49±2.00^{a1}	(n = 6) 10.64±1.09^{a1}	(n = 6) 10.46±0.95^{a1}
	H_2	(n = 6) 10.71±1.52^{a1}	(n = 6) 10.11±1.87^{a1}	(n = 6) 9.83±0.98^{a1}	(n = 6) 9.16±1.72^{a1}
	H_3	(n = 6) 11.80±1.73^{a1}	(n = 6) 10.41±1.05^{ab1}	(n = 6) 9.55±0.74^{b1}	(n = 6) 10.26±1.15^{b1}
Proteins (%)	H_1	(n = 6) 10.34±0.53^{a1}	(n = 6) 10.89±0.38^{b1}	(n = 6) 10.90±0.48^{b1}	(n = 6) 11.01±0.32^{b1}
	H_2	(n = 6) 10.34±0.58^{a1}	(n = 6) 10.86±0.24^{b1}	(n = 6) 10.98±0.34^{b1}	(n = 6) 11.20±0.47^{b1}
	H_3	(n = 6) 10.40±0.61^{a1}	(n = 6) 10.88±0.56^{b1}	(n = 6) 11.30±0.19^{b1}	(n = 6) 11.03±0.56^{b1}
Salt (%)	H_1	(n = 6) 1.91±0.43^{a1}	(n = 6) 1.84±0.38^{a1}	(n = 6) 1.77±0.24^{a1}	(n = 6) 1.74±0.57^{a1}
	H_2	(n = 6) 1.99±0.36^{a1}	(n = 6) 1.82±0.66^{a1}	(n = 6) 1.70±0.49^{a1}	(n = 6) 1.65±0.47^{a1}
	H_3	(n = 6) 1.73±0.21^{a1}	(n = 6) 1.77±0.38^{a1}	(n = 6) 1.56±0.22^{a1}	(n = 6) 1.48±0.22^{a1}
N (%)	H_1	(n = 4) 90.22±1.85^{a1}	(n = 6) 93.98±0.84^{b1}	(n = 5) 93.98±1.13^{b1}	(n = 6) 94.33±0.71^{b1}
	H_2	(n = 4) 89.48±2.02^{a1}	(n = 4) 92.28±0.91^{b2}	(n = 6) 93.67±0.48^{b1}	(n = 6) 93.67±0.84^{b12}
	H_3	(n = 3) 87.43±2.76^{a1}	(n = 6) 92.75±0.79^{b2}	(n = 5) 93.22±0.52^{b1}	(n = 6) 92.95±0.76^{b2}
O_2 (%)	H_1	(n = 4) 1.36±0.18^{a1}	(n = 6) 0.90±0.35^{a1}	(n = 5) 0.95±0.92^{a1}	(n = 6) 0.24±0.37^{b1}
	H_2	(n = 4) 1.12±0.27^{a1}	(n =4) 0.59±0.11^{b12}	(n = 6) 0.17±0.12^{c2}	(n = 6) 0.13±0.11^{c12}
	H_3	(n = 3) 1.08±0.43^{a1}	(n =6) 0.42±0.20^{b2}	(n = 5) 0.11±0.11^{c2}	(n = 6) 0.06±0.03^{c2}
$C0_2$ (%)	H_1	(n = 4) 8.42±1.89^{a1}	(n = 6) 5.15±0.59^{b1}	(n = 5) 5.02±0.56^{b1}	(n = 6) 5.42±0.62^{b1}
	H_2	(n = 4) 9.55±2.14^{a1}	(n = 4) 7.12±0.88^{b2}	(n = 6) 6.18±0.40^{b2}	(n = 6) 6.15±0.82^{b12}
	H_3	(n = 3) 11.05±2.35^{a1}	(n = 6) 6.82±0.81^{b2}	(n = 5) 6.72±0.44^{b2}	(n = 6) 7.00±0.72^{b2}

Values in the same row with different superscript letter are statistically different (P<0.05). Values in the same column with different superscript number are statistically different (P<0.05).

spp. grew from the initial contamination of ca. two log_{10} cfu/g to as high as 7 log_{10} cfu/g after 21 days of refrigerated storage (Pala *et al*., 2016; Spanu *et al.,* 2017, 2018). The aerobic mesophilic bacteria from ca. four log_{10} cfu/g observed at the day of packaging decreased to <3 log_{10} cfu/g after fifteen days of storage, then slowly increased (ca. 0.5 log_{10} cfu/g) at 45 days to levels always below the initial contamination. These results are different as compared to other sheep ricotta cheeses. In MAP fresh ricotta cheese aerobic mesophilic count increased from levels <3 log_{10} cfu/g up to 7-8 log_{10} cfu/g after 21 days of refrigerated storage, largely accountable to the growth of psychotropic microorganisms such as *Pseudomonas* spp. (Pala *et al.,* 2016 Spanu *et al*., 2017). This difference is explained by the absence in the *novel smoked ricotta cheese* of *Pseudomonas* spp. contamination, further indication that GHP were strictly observed during the manufacturing process. In traditional salted ricotta cheese (*i.e. ricotta toscanella*) the mean aerobic mesophilic bacteria count on the rind surface was ca. 7-8 log_{10} cfu/g before vacuum packaging (Spanu *et al.,* 2013; Casti *et al.,* 2016). Higher counts of salted ricotta are justified by the phases of pressing, dry salting and maturation conducted with the exposed product which increase the risk of cross contamination originating from the processing environment (Ibba *et al.,* 2013). A progressive reduction of O_2% concentration in the headspace was observed during storage, indicating the growth of aerobic microorganism, mostly yeast and molds. Instead, the reduction in CO_2% concentration is the results of gas solving in the product. MAP packaging is used in fresh ricotta cheese, showing an initial increase of O_2% concentration, followed by a successive decrease (Pala *et al*., 2016; Spanu *et al.,* 2017, 2018). This could be explained with the different size of fresh ricotta compared to *NS-Ricotta Cheese* (1.1-1.7 kg vs. 300 g) and therefore the less favorable headspace/food ratio. The greater amount of O_2 incorporated in the *ricotta fresca* food matrix, which is successively released into the headspace, greatly impact on the time needed to reach the equilibrium condition of the gas mixture (Simpson and Carevic, 2004).

Conclusions

In the frame of an increasing globalization of food market, innovation is an essential strategy for small and medium sized enterprises to achieve a competitive advantage versus global foods (Murphy, 2002; Avermaete *et al.,* 2004; Gellynck *et al.,* 2007; Albayrak and Gunes, 2010). However, conjugate innovation and tradition is a complex task due to the controversy involved (Jordana, 2000). The present study demonstrated the feasibility of introducing product innovation in a traditional dairy product of Sardinia such as *ricotta cheese*. Despite the microbiological results obtained in the present study indicate a general safety of the product, definitive conclusion should be supported by specific challenge test. In fact, the intrinsic properties describe a product supporting the possible growth of spoilage and pathogens microorganism. Under the defined refrigerated storage conditions, psychotropic microorganisms such as *Pseudomonas* spp, *Listeria monocytogenes* and eventual *B. cereus* psychotropic strains represent major

Table 5. pH, a_W, physico-chemical and headspace gas composition ($\bar{x}$ ±SD) of ricotta samples by smoking time (1, 2 and 3 hours) stored at 7°C.

Parameters	Smoking	Day of storage			
		T_0	T_{15}	T_{30}	T_{45}
pH	H_1	(n = 6) 6.32±0.03^{a1}	(n = 6) 6.26±0.12^{a1}	(n = 6) 6.10±0.09^{b1}	(n = 6) 6.14±0.06^{b1}
	H_2	(n = 6) 6.29±0.03^{a1}	(n = 6) 6.15±0.22^{a1}	(n = 6) 5.91±0.68^{b2}	(n = 6) 5.91±0.11^{b2}
	H_3	(n = 6) 6.32±0.06^{a1}	(n = 6) 6.07±0.24^{b1}	(n = 6) 5.71±0.86^{c3}	(n = 6) 5.81±0.16^{c2}
a_w	H_1	(n = 6) 0.984±0.004^{a1}	(n = 6) 0.979±0.006^{a1}	(n = 6) 0.979±0.006^{a1}	(n = 6) 0.982±0.006^{a1}
	H_2	(n = 6) 0.984±0.005^{a1}	(n = 6) 0.981±0.006^{a1}	(n = 6) 0.982±0.004^{a1}	(n = 6) 0.984±0.005^{a1}
	H_3	(n = 6) 0.986±0.005^{a1}	(n = 6) 0.980±0.003^{ab1}	(n = 6) 0.979±0.004^{b1}	(n = 6) 0.985±0.006^{ab1}
Moisture (%)	H_1	(n = 6) 75.08±1.17^{a1}	(n = 6) 75.61±1.86^{a1}	(n = 6) 75.95±.06^{a1}	(n = 6) 74.99±2.14^{a1}
	H_2	(n = 6) 74.92±2.29^{a1}	(n = 6) 73.72±2.03^{a1}	(n = 6) 75.03±1.79^{a1}	(n = 6) 75.12±1.98^{a1}
	H_3	(n = 6) 73.66±1.92^{a1}	(n = 6) 73.66±1.15^{a1}	(n = 6) 73.41±2.17^{a1}	(n = 6) 74.37±1.83^{a1}
Fat (%)	H_1	(n = 6) 10.89±1.01^{a1}	(n = 6) 10.15±1.83^{a1}	(n = 6) 9.65±1.75^{a1}	(n = 6) 10.38±0.89^{a1}
	H_2	(n = 6) 10.71±1.52^{ab1}	(n = 6) 10.92±1.09^{b1}	(n = 6) 9.78±0.87^{ab1}	(n = 6) 9.38±1.76^{a1}
	H_3	(n = 6) 11.80±1.73^{a1}	(n = 6) 10.68±1.00^{ab1}	(n = 6) 10.43±1.29^{ab1}	(n = 6) 9.66±0.51^{b1}
Proteins (%)	H_1	(n = 6) 10.34±0.53^{a1}	(n = 6) 10.82±0.37^{ab1}	(n = 6) 10.93±0.45^{b1}	(n = 6) 11.14±0.34^{b1}
	H_2	(n = 6) 10.34±0.58^{a1}	(n = 6) 10.79±0.44^{ab1}	(n = 6) 10.97±0.17^{b1}	(n = 6) 11.16±0.18^{b1}
	H_3	(n = 6) 10.40±0.61^{a1}	(n = 6) 11.06±0.34^{b1}	(n = 6) 11.09±0.35^{b1}	(n = 6) 11.31±0.37^{b1}
Salt (%)	H_1	(n = 6) 1.91±0.43^{a1}	(n = 6) 1.70±0.53^{a1}	(n = 6) 1.79±0.40^{a1}	(n = 6) 1.69±0.69^{a1}
	H_2	(n = 6) 1.99±0.36^{a1}	(n = 6) 1.82±0.66^{a1}	(n = 6) 1.70±0.49^{a1}	(n = 6) 1.65±0.47^{a1}
	H_3	(n = 6) 1.73±0.21^{a1}	(n = 6) 1.66±0.42^{a1}	(n = 6) 1.49±0.33^{a1}	(n = 6) 1.44±0.34^{a1}
N (%)	H_1	(n = 4) 90.22±1.85^{a1}	(n = 6) 93.63±0.94^{b1}	(n = 6) 93.80±2.56^{b1}	(n = 6) 92.27±3.44^{ab1}
	H_2	(n = 4) 89.48±2.02^{a1}	(n = 6) 93.03±0.77^{b1}	(n = 6) 93.53±0.83^{b1}	(n = 6) 93.40±0.44^{b1}
	H_3	(n = 3) 87.43±2.76^{a1}	(n = 6) 92.67±0.81^{b1}	(n = 6) 92.73±0.59^{b1}	(n = 6) 92.52±1.01^{b1}
O_2 (%)	H_1	(n = 4) 1.36±0.18^{a1}	(n = 6) 0.70±0.21^{b1}	(n = 6) 0.12±0.14^{cb1}	(n = 6) 0.01±0.01^{c1}
	H_2	(n = 4) 1.12±0.27^{a1}	(n =6) 0.69±0.58^{a1}	(n = 6) 0.18±0.09^{b1}	(n = 6) 0.09±0.06^{b2}
	H_3	(n = 3) 1.08±0.43^{a1}	(n =6) 0.28±0.14^{b1}	(n = 6) 0.08±0.06^{b1}	(n = 6) 0.09±0.04^{b2}
$C0_2$ (%)	H_1	(n = 4) 8.42±1.89^{a1}	(n = 6) 5.68±1.03^{b1}	(n = 6) 6.01±2.61^{b1}	(n = 6) 7.60±3.54^{b1}
	H_2	(n = 4) 9.55±2.14^{a1}	(n = 6) 5.87±0.37^{b1}	(n = 6) 6.32±0.75^{b1}	(n = 6) 6.47±0.40^{b1}
	H_3	(n = 3) 11.05±2.35^{a1}	(n = 6) 6.98±0.71^{b2}	(n = 6) 7.27±0.57^{b1}	(n = 6) 7.04±1.04^{b1}

Values in the same row with different superscript letter are statistically different (P<0.05). Values in the same column with different superscript number are statistically different (P<0.05).

concerns. The salted and smoked ricotta cheese is a food that should be regarded at high risk of secondary contamination; therefore, it is essential the strict implementation of good hygienic practices especially in the production steps between curd rising and packaging.

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