



X-ray irradiation as a valid technique to prolong food shelf life: The case of ricotta cheese

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ARTICLE INFO

Article history:

Received 18 April 2019

Received in revised form

25 July 2019

Accepted 26 July 2019

Available online 10 August 2019

ABSTRACT

The sanitising effects of X-rays were studied on ricotta cheese at intensities of 0.5, 2 and 3 kGy, using products manufactured artisanally and industrially. Microbiological, sensory and pH evaluations were performed during refrigerated storage. The artisanal ricotta irradiated at the two highest intensities (2 and 3 kGy) remained acceptable for more than 20 days, whereas the untreated samples became unacceptable after only 3 days of storage. The shelf life of the product irradiated at 0.5 kGy was limited to 14 days, due to the appearance of sensory defects. The industrial product irradiated at all X-rays intensities recorded a significant shelf life prolongation up to 84 days compared with the control, which was rejected after 40 days due to sensory defects. The results show that X-ray treatment can significantly prolong the shelf life of ricotta cheese, boosting the marketability of this fresh dairy product far from the local production sites.

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1. Introduction

Fresh dairy products are characterised by short shelf lives, being an excellent growth medium for a wide range of microorganisms (Quigley et al., 2013). Ricotta cheese, in particular, has traditionally been prepared by heating whey and acidifying the hot liquid with lactic acid to coagulate whey proteins (Di Piero, Sorrentino, Mariniello, Giosafatto, & Porta, 2011; Modler & Emmons, 2001; Pizzillo, Claps, Cifuni, Fedele, & Rubino, 2005). The coagulated curd mass floats to the surface, is scooped off and transferred into perforated trays to drain the exhausted whey (Modler & Emmons, 2001).

Fresh ricotta has high moisture content, high concentration of residual sugars, initial pH above 6.0 and does not need any starter culture addition in production. As a consequence, fresh ricotta has limited shelf life (2–3 days) even under refrigeration (Hough, Puglieso, Sanchez, & Mendes da Silva, 1999; Martins, Cerqueira, Souza, Carmo Avides, & Vincente, 2010). Due to naturally poor competitive microflora (Pintado, Macedo, & Malcata, 2001),

composition, inherent physical and chemical properties and absence of preservatives, fresh ricotta is an excellent substrate for the growth of spoilage micro-organisms mainly represented by *Pseudomonas* spp., yeasts, moulds and Enterobacteria (De Santis & Mazzette, 2002; Pala et al., 2016; Pintado et al., 2001).

Whey products processed at high temperatures and successively cooled and stored under refrigerated conditions are particularly exposed to the risk of *Bacillus cereus* growth (Heyndrickx & Scheldeman, 2002). The endospores are activated by the heat treatment applied to denature whey proteins ($T > 80$ °C) and their growth is facilitated by the absence of a competing microbiota, inactivated by the heat treatment (Scheldeman, Herman, Foster, & Heyndrickx, 2006). *B. cereus* psychrotropic strains can grow at temperature as low as 4–5 °C and the risk of growth is enhanced by slow cooling of the product: a temperature lower than 10 °C should be reached in a short time, being the interval of temperatures between 40 and 10 °C favourable to *B. cereus* growth (Huck, Hammond, Murphy, Woodcock, & Boor, 2007). *B. cereus* can enter the dairy chain mainly through raw milk contaminated at farm level (Heyndrickx, 2011). However, contamination may also arise from the food-processing environment (da Silva Fernandes, Fujimoto, Schneid, Kabuki, & Kuaye, 2014). All these bacteria have negative impact on texture, appearance, colour, odour and taste of

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cheese (Baruzzi, Lagonigro, Quintieri, Morea, & Caputo, 2012; Cantoni, Stella, Cozzi, Iacumin, & Comi, 2003; Caputo et al., 2015).

The improvement of hygienic practices is a measure that could certainly reduce the level of initial contamination of ricotta surface. However, artisanal ricotta shelf life is very short (much less than one week). For this reason, at an industrial level ricotta cheese is generally heat-treated. The industrial process includes a final pasteurisation step, which assures a shelf life ranging between 20 and 40 days (Mucchetti & Neviani, 2006). Depending on the food business operator, fresh ricotta can be packed under atmospheric air or modified atmosphere conditions (MAP) (30% CO₂ and 70% N₂) (Spanu et al., 2018).

The further shelf life prolongation of both artisanal and industrial cheese represents a key factor for a wider ricotta market at both local and international level, thus justifying the continued search for novel preservation strategies. Conventional thermal technologies, nevertheless effective, may cause negative changes in nutritional value, flavour, colour or texture of final product, or may lead to create undesirable by-products that might eventually affect food quality and safety (Li & Farid, 2016; Pereira & Vicente, 2010). The use of bio-preservatives (i.e., nisin, other bacteriocins, natural active compounds or bioprotective cultures), alone or combined with MAP, has been proposed to compete with contaminants and to preserve quality and safety (Sobrino-López & Martín-Belloso, 2008).

Nowadays consumers prefer more natural and healthy foods, thus pushing researchers to non-thermal sanitisation techniques such as pulsed electric fields (PEF), pulsed light (LP), irradiation (Pan & Han, 2017), high hydrostatic pressures (HHP), cold plasma, ultrasound, etc. The adoption of unconventional technologies for food preservation could be an alternative strategy (Roohinejad, Koubaa, Sant'Ana, & Greiner, 2018), even though it is possible that these treatments can lead to undesirable changes that request dedicated studies.

Gamma rays, electron beam and X-rays also have non-thermal mechanism of action (Farkas, 2006). In particular, X-rays are generated by ionising radiation of mechanical origin without any radioactive substance in the system and with the possibility to pass through thick materials (approximately 30–40 cm). This feature makes this technology suitable for the treatment of already packaged foods, avoiding recontamination of the product. According to a good practice of irradiation, the application of X-rays must never exceed the recommended dose of 10 kGy (Roberts, 2016).

Each country has a different approach to food irradiation, uses different labels, allows different doses of irradiation; generally, the regulations comply with the general standard of Codex for irradiated foods (Roberts, 2003, 2016). More than 60 countries have approved the use of irradiation. Ionising radiation inactivates microorganisms, damaging the critical elements of the cell, more often DNA and RNA. This cell damage is the result of the direct or indirect effect of the radiant energy, thus preventing multiplication and most of the cellular functions (Liberty, Dickson, Achebe, & Salihu, 2013). Interestingly, food irradiation has been shown to inactivate not only spoilage microorganisms but also pathogens in food of dairy sector, fish and vegetables (Bougle & Stahl, 1994; Kim et al., 2010; Lacivita et al., 2019; Mahmoud, 2010; Mahmoud, Nannapaneni, Chang, Wu, & Coker, 2016; Tsiotsias, Savvaidis, Vassila, Kontominas, & Kotzekidou, 2002), but to our knowledge no data are available for fresh ricotta cheese.

Therefore, the purpose of the present study was to investigate X-rays treatment of artisanal and industrial fresh ricotta cheese. The efficacy of this treatment to prolong the shelf life of the product was evaluated in terms of microbiological and sensory quality of packaged samples after different treatments.

2. Materials and methods

2.1. X-ray treatment

The X-ray treatments on fresh ricotta cheese samples were performed using the RS-2400 system (Rad Source, Brentwood, TN, USA) installed at the IZS Institute (Foggia, Italy). The first test was performed on 50 samples of fresh artisanal ricotta cheese (50 g), purchased from the company “Caseificio della Daunia” in Foggia (Italy). On the same day of production, the samples were packed individually (PA/PE type 95 bags) and transported to the IZS Institute under refrigeration conditions. The second test was carried out on 80 pasteurised industrial packaged ricotta samples (100 g), kindly provided by Granarolo S.p.A. (Bologna, Italy). All the samples, one day after production, underwent X-ray irradiation treatment at 0.5, 2 and 3 kGy, respectively. Untreated control samples and treated samples in both tests were stored at 4 °C for 24 and 84 days, for artisanal and industrial samples, respectively.

2.2. Microbiological analyses

In each test, the microbiological quality of control and treated samples was analysed by monitoring microbial growth of mesophilic bacteria, *Pseudomonas* spp., Enterobacteriaceae, yeasts and *B. cereus*. For artisanal ricotta cheese, the sampling was carried out at time 0, 1 and every 2/3 days up to 24 days of storage, while the industrial samples were analysed at time 0, 1, every 7 days up to 56 days and at 84 days of storage. To this aim, twenty grams of ricotta cheese were removed from each pack, diluted with 200 mL of 0.9% NaCl solution and thoroughly homogenised (Bag Mixer Interscience, St Nom, France) before making decimal dilutions of homogenates. The dilutions were plated on appropriate media in Petri dishes. The classic plate count technique was applied. Culture media and incubating conditions for spoilage microorganisms were reported in the study of Del Nobile, Gammariello, Conte, and Attanasio (2009), also dealing with ricotta cheese. *B. cereus* counts were determined using MYP culture medium (Oxoid, Milan, Italy), adding, after sterilisation, egg yolk emulsion (50 mL, Oxoid) and Polymyxin B (1 bottle, SR009E, Oxoid) as supplements. The fitting of experimental data allowed quantifying the microbiological acceptability limit (MAL), calculated according to the same mathematical approach adopted by Angiolillo, Conte, Faccia, Zambriani, and Del Nobile (2014). The pH value was assessed on homogenates of ricotta cheese, in duplicate, by a pH-meter (Crison GLP 21+, Barcelona, Spain), after appropriate calibration of the instrument (Lacivita et al., 2016).

2.3. Sensory analysis

Sensory analysis was performed by 7 trained panellists from the Food Packaging Laboratory of the University of Foggia. They had several years of experience in sensory evaluation, but before the analysis they were re-trained (2 sessions, 2 h each session) to better define sensory attributes and score evaluation. To this aim, odour, colour, texture and global quality were taken into account, using properly evaluation grid ranged from 0 to 7, where 4 represented the cheese acceptability threshold (Chen, Wolle, & Sommer, 2009; Lacivita et al., 2016). The fitting of experimental data allowed quantifying the sensory acceptability limit (SAL), as reported in Angiolillo et al. (2014).

2.4. Statistical analysis

Both microbiological, pH determination and sensory analyses were carried out twice, on two different samples treated and

packaged separately. Mean and standard deviation of experimental data were calculated. Fitting of experimental data gave us MAL and SAL parameters. These fitting parameters were compared by one-way ANOVA test (STATISTICA 7.1 for Windows, StatSoft Inc., Tulsa, OK, USA). To determine significant differences among samples the Duncan's multiple range test, with the option of homogeneous groups ($p < 0.05$), was used. The lowest value among the MAL and SAL parameters gave us the final shelf life.

3. Results and discussions

This study investigated the effect of three X-ray irradiation doses (0.5, 2 and 3 kGy) on microbial spoilage and sensory quality of fresh ricotta cheese over time when kept under refrigeration. In particular, two tests were carried out to evaluate the effectiveness of X-rays on both artisanal and commercial fresh products.

3.1. Effects of X-rays on microbiological quality of artisanal and industrial fresh ricotta cheese

To evaluate the X-ray effects on microbial population of fresh ricotta, *Pseudomonas* spp., Enterobacteriaceae, yeasts, total mesophilic count and *B. cereus* were monitored during time, as main responsible factors for product stability (Pala et al., 2016; Sattin et al., 2016; Spanu et al., 2017, 2016). Fig. 1 shows the evolution of *Pseudomonas* spp. in treated samples of artisanal ricotta compared with non-irradiated control cheese (Ctrl). As can be seen, for the irradiated samples the applied treatment exhibited different effects on microbial counts. In particular, for both samples irradiated at 2 and 3 kGy *Pseudomonas* spp. growth was completely inhibited by the X-ray treatment. On the contrary, the samples of artisanal ricotta irradiated at 0.5 kGy showed a long lag phase, little more than one week, and then slightly increased, without ever reaching the microbiological acceptability limit set to 10^6 cfu g⁻¹. The microbial evolution of irradiated samples was significantly different compared with non-irradiated control cheese. In fact, as can be seen in Fig. 1, a gradual increase of *Pseudomonas* spp. population was noticed during the 10 days of storage monitored, overcoming the microbiological acceptability limit after only 3 days.

It is well known that *Pseudomonas* spp. are psychrotrophic bacteria with a short generation time (only a few hours) at refrigeration temperature. As a consequence, microbial cell counts can exceed 10^6 cfu g⁻¹ within a few days of chilled storage (Samaržija, Zamberlin, & Pogacic, 2012). This was, in fact, the behaviour

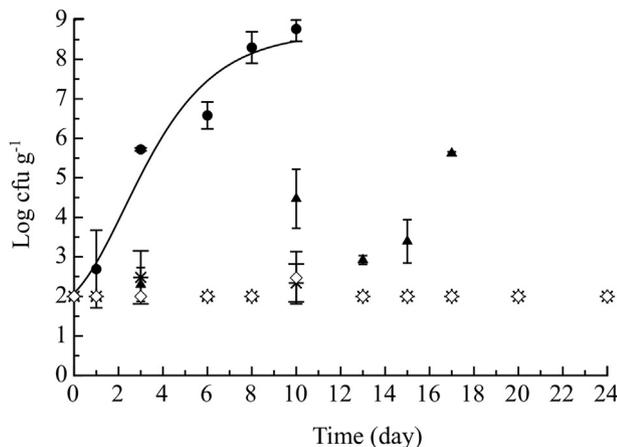


Fig. 1. Evolution of *Pseudomonas* spp. in X-ray treated artisanal Ricotta cheese (\blacktriangle , 0.5 kGy; $*$, 2 kGy; \diamond , 3 kGy) compared with non-irradiated control (\bullet); symbols are the experimental data, line is best fit to control data.

observed in the control samples, whereas in the treated products the higher doses of treatment were able to keep the microbial count very low during the 24 days of monitoring. These results were well supported by an earlier study (Lacivita et al., 2019), where the effects of X-ray were assessed on Fiordilatte cheese.

A very similar behaviour was also observed for Enterobacteriaceae and yeasts. Figs. 2 and 3, respectively, show the evolution of Enterobacteriaceae and yeasts of X-ray treated artisanal ricotta samples compared with the control cheese. As can be seen, for both microbial groups the X-ray treatment significantly reduced the microbial growth. Contrary to this trend, the microbial count of Enterobacteriaceae and yeasts in the control samples gradually increased, overtaking the microbial acceptability limit set to 10^4 and 10^6 cfu g⁻¹ after 4 and 8 days, respectively. According to other studies also reported in the literature (Konteles, Sinanoglou, Batrinou, & Sflomos, 2009; Odueke, Farag, Baines, & Chadd, 2016), the initial microbial load of total mesophilic count (10^5 cfu g⁻¹) was significantly reduced immediately after irradiation by more than 3 log cycles in treated ricotta while it increased in the control cheese. These great differences between control and treated products were maintained during the entire monitoring period; samples treated at 2 and 3 kGy maintained a microbial count around 10^3 cfu g⁻¹, whereas samples treated at 0.5 kGy reached about 10^6 cfu g⁻¹, and control ricotta arrived to about 10^9 cfu g⁻¹. The irradiation treatment, even at low doses, exerted a great impact on total mesophilic counts. *B. cereus* was not detected in both non-irradiated and treated samples. In general, the experimental findings on artisanal ricotta cheese confirmed the effectiveness of irradiation already verified on other types of fresh dairy products (Badr, 2011; Huo, Bai, Guo, & Zhao, 2013; Lacivita et al., 2019; Velasco, Cambero, Ordóñez, & Cabeza, 2019) and suggested that with 2 kGy treatment a total spoilage control of artisanal Ricotta can be reached.

Regarding the microbiological quality of industrial ricotta samples, it was striking to find no spoilage proliferation, in terms of *Pseudomonas* spp., Enterobacteriaceae, yeasts and *B. cereus*, in all the cheese samples examined during a long storage time. For mesophilic bacteria (data not shown) the same trends recorded for control and treated artisanal samples, respectively, were also found for industrial ricotta cheese. The lack of spoilage of these samples was due to the stabilising thermal treatment applied just before packaging that generally assures a shelf life of more than one month. Therefore, for the industrial ricotta, the effects of X-ray treatments were more evident in terms of sensory quality, as reported below.

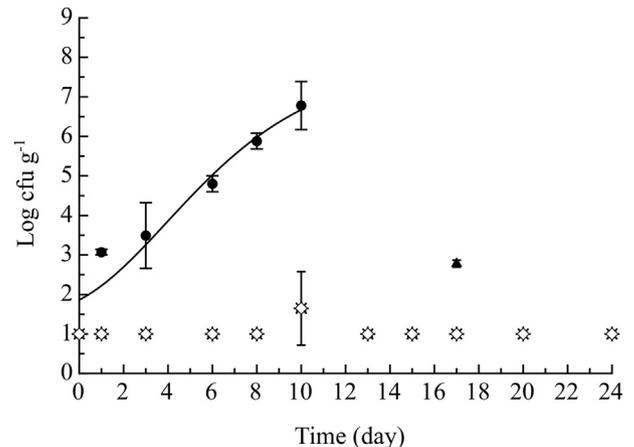


Fig. 2. Evolution of Enterobacteriaceae of X-ray treated artisanal Ricotta cheese (\blacktriangle , 0.5 kGy; $*$, 2 kGy; \diamond , 3 kGy) compared with non-irradiated control (\bullet); symbols are the experimental data, line is best fit to control data.

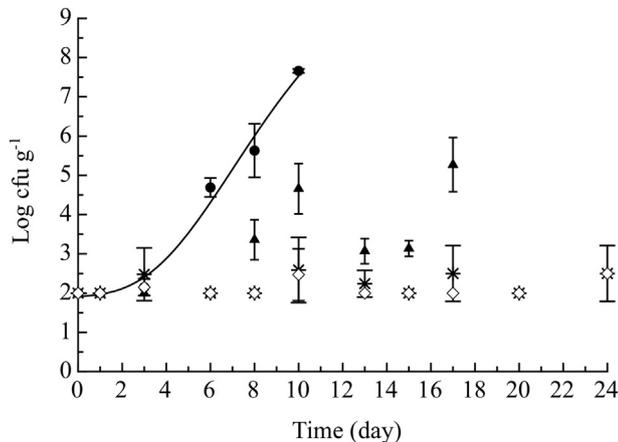


Fig. 3. Evolution of yeasts of X-ray treated artisanal Ricotta cheese (▲, 0.5 kGy; *, 2 kGy; ◆, 3 kGy) compared with non-irradiated control (●); symbols are the experimental data, line is best fit to control data.

No great pH variations in both artisanal and industrial treated ricotta samples were found, in line with other studies where irradiation was applied to fresh dairy cheese (Badr, 2011; Konteles et al., 2009). Specifically, in the artisanal products pH ranged around 7.20 with very similar trends among samples during the entire period. On the other side, a significant pH decline was recorded in the control cheese just after the first 3 days of storage (from 7.20 to 6.70). Likewise, in the industrial dairy products, a great difference between treated and control ricotta was found. In this case, the pH of treated samples ranged around 6.30, whereas, pH in the control cheese slightly decreased reaching values that accounted for about 5.75 when the product became unacceptable (after circa 40 days).

3.2. Effects of X-rays on the sensory quality of artisanal and industrial fresh ricotta cheese

For the sensory evaluation the trained panel judged colour, odour and texture of control and irradiated artisanal fresh cheese. Trend of mean values of sensory scores were shown in Fig. 4 in terms of overall quality. As can be inferred from the figure, non-irradiated samples became unacceptable after 6 days of storage, cheese irradiated at 0.5 kGy after 14 days, whereas the two samples

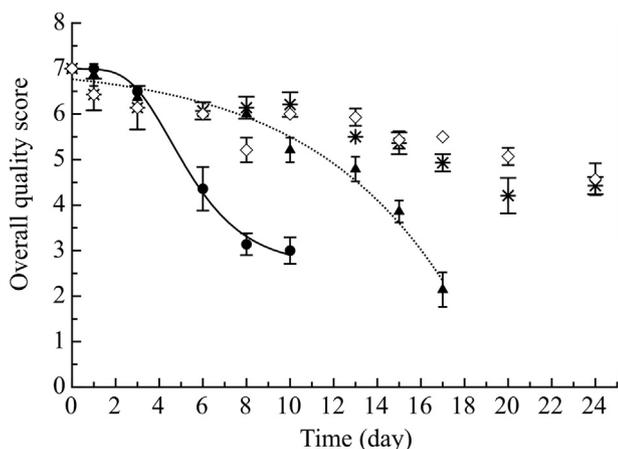


Fig. 4. Evolution of overall quality of irradiated artisanal Ricotta cheese samples (▲, 0.5 kGy; *, 2 kGy; ◆, 3 kGy) compared with non-irradiated control (●); symbols are the experimental data, solid and dotted lines are best fit to control and 0.5 kGy data, respectively.

treated at higher doses remained acceptable for 24 days with a very similar trend. As expected, sensory evolution can be strictly correlated to microbial proliferation, thus confirming that in cheese samples where microbial growth was inhibited (ricotta treated at 2 and 3 kGy) also sensory properties were more appreciated (Lacivita et al., 2019).

As regards industrial ricotta sensory quality, important and interesting differences between control and treated samples were found, elongating the storage period to 84 days. As reported in Fig. 5, the kinetic decay of the overall quality referred to the control samples was very different from that related to treated cheese. In particular, while the control ricotta became unacceptable after 38 days of storage, all the X-ray treated samples remained acceptable for more than 80 days, without any substantial differences among the three irradiation-doses.

These experimental findings in terms of sensory quality on both artisanal and industrial ricotta also suggested that X-rays did not compromise main ricotta attributes, even when applied at high dose. The same result was also recorded by Aly, Farag, and Galal (2012), who found no differences between treated and untreated cheese samples after e-beam and γ -irradiation at different doses (from 1 to 5 kGy). However, in this context, data from scientific literature of irradiated food are dependent on the type of irradiation and on the nature of dairy cheese. Sometimes, some changes, especially in odour and colour, were perceived after ionising radiation at dose up to 3 kGy (Konteles et al., 2009; Velasco et al., 2019; Velasco, Ordóñez, Cabeza, Hoz, & Cambero, 2016).

3.3. Shelf life of artisanal and industrial ricotta cheese

In general, the shelf life of food products is compromised by undesired spoilage microorganism proliferation and consequently by sensory deterioration (Lacivita et al., 2019). Therefore, also in the current study, the shelf life of tested ricotta cheese samples was reported in Table 1 as the lowest value among microbial acceptability limits, in terms of MAL^{Pseudomonas}, MAL^{Enterobacteriaceae} and MAL^{Yeasts}, and sensory acceptability limits in terms of overall quality (SAL). As expected, a substantial difference between artisanal and industrial ricotta was found, being that the industrial product was characterised by a longer shelf life than the artisanal ricotta (Mucchetti & Neviani, 2006). As a fact, the factors that affect ricotta quality are different between artisanal and industrial cheese and consequently the X-rays treatment influenced the two types of

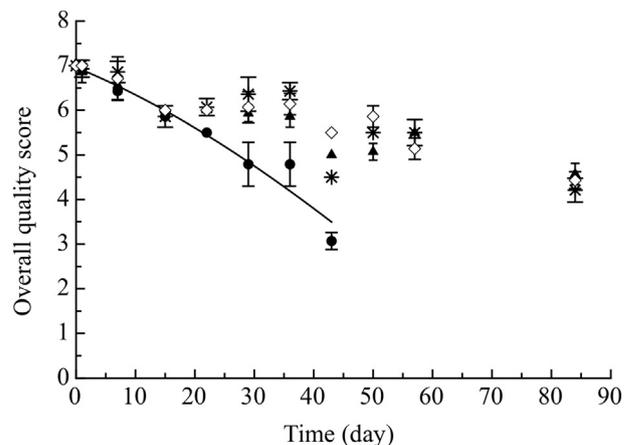


Fig. 5. Evolution of overall quality of irradiated industrial Ricotta cheese samples (▲, 0.5 kGy; *, 2 kGy; ◆, 3 kGy) compared with non-irradiated control (●); symbols are the experimental data, line is best fit to control data.

Table 1

Shelf-life (days) of artisanal and industrial Ricotta cheese as the lowest value between microbiological acceptability limit (MAL) for *Pseudomonas*, Enterobacteriaceae and yeasts and sensory acceptability limit (SAL).^a

Samples	Microbial quality (days)			SAL (days)	Shelf life (days)
	MAL ^{<i>Pseudomonas</i>}	MAL ^{Enterobacteriaceae}	MAL ^{Yeasts}		
Artisanal					
Ctrl	3.92 ± 0.61 ^a	4.23 ± 0.87 ^a	7.99 ± 0.41 ^a	6.42 ± 0.22 ^a	3.92 ± 0.61 ^a
0.5 kGy	>17	>17	>17	14.23 ± 0.44 ^b	14.23 ± 0.44 ^b
2 kGy	>24	>24	>24	>24	>24
3 kGy	>24	>24	>24	>24	>24
Industrial					
Ctrl	>43	>43	>43	37.94 ± 0.11 ^a	37.94 ± 0.11 ^a
0.5 kGy	>84	>84	>84	>84	>84
2 kGy	>84	>84	>84	>84	>84
3 kGy	>84	>84	>84	>84	>84

^a Values in columns with different superscript letters are significantly different ($p < 0.05$).

products differently. Specifically, control artisanal cheese remained acceptable for a few days (little more than 3), due to prompt *Pseudomonas* spp. proliferation. For artisanal samples treated at 0.5 kGy, a shelf life prolongation by about 5 times was recorded: the samples remained acceptable for about 2 weeks. X-rays significantly inhibited the cheese spoilage, but from the sensory point of view after 14 days some undesirable colour changes appeared on the product, thus determining the end of shelf life. Ricotta treated at 2 or 3 kGy recorded a shelf life 8 times longer than the control cheese, thus demonstrating the great effect of high doses of X-rays irradiation on both microbiological and sensory quality for a prolonged storage period. Shelf life of industrial product was mainly due to sensory deterioration because in both control and treated samples no significant spoilage growth was detected. Control cheese, even if acceptable from the microbiological point of view, became unacceptable after less than 40 days due to anomalous superficial colour appearance. On the contrary, treated industrial ricotta remained acceptable from both the microbiological and sensorial points of view for the entire monitoring period, thus recording a shelf life that was doubled (84 days) compared with the corresponding control cheese. On the basis of our findings X-rays can represent a useful treatment to extend the shelf life of both artisanal and industrial fresh Ricotta cheese.

4. Conclusions

X-rays were successfully tested on artisanal and industrial ricotta. Specifically, for the artisanal product, the shelf life of the sample treated at 0.5 kGy reached 14 days of storage, compared with 3 days for the untreated sample. In comparison, samples treated at 2 and 3 kGy remained acceptable for more than 20 days. The industrial product, being subjected to thermal pasteurisation, reached a shelf life of about 40 days even without any treatment. Treated samples recorded a significant shelf life prolongation, up to 84 days without any defect from the microbiological and sensory point of view. This study highlights the great sanitising power of X-rays and the different effects according to the initial quality of the product. An intensity of 2 kGy for both artisanal or industrial ricotta cheese is enough to obtain excellent results.

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