

Use of E-beam radiation to eliminate *Listeria monocytogenes* from surface mould cheese

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Summary. Camembert and Brie soft cheese varieties were subjected to E-beam irradiation as a sanitation treatment. The effects of treatments on microbiota and selected physicochemical properties were also studied. The absorbed doses required to meet the food safety objective (FSO) according to EU and USDA criteria for *Listeria monocytogenes* were 1.27 and 2.59 kGy, respectively. The bacterial load, mainly lactic acid bacteria, was reduced by the treatment but injured cells were recovered during storage at 14°C. The radiation treatment gave rise to negligible changes in the pH and water activity at doses required to achieve microbial safety. [Int Microbiol 2015; 18(1):33-40]

Keywords: *Listeria monocytogenes* · food safety objective (FSO) · soft mould-ripened cheeses · E-beam radiation

Introduction

Camembert and Brie cheeses are included in the soft cheeses types. They are manufactured by a similar technology and the main difference between them is the diameter size, i.e., 10–11 cm and 22–36 cm for Camembert and Brie, respectively [13]. The main feature of both cheeses is that, after the lactic acid fermentation, a white crust is formed due to the growth of *Penicillium camemberti* in the surface. Due to the mould activity, the ripening is very fast at room temperature and to decelerate this phenomenon the cheese is commonly stored under refrigeration.

Since *Listeria monocytogenes* is a psychrotrophic organism, it may grow if it is present.

Listeria monocytogenes is the causative agent of a disease that may be acquired by food ingestion. Several *L. monocytogenes* outbreaks have been reported due to soft cheeses [11,20,30,53]. This bacterium can reach the product mainly from environmental contamination or, more rarely, bovine mastitis [7,15,45]. It has been isolated from cheeses made from raw, low heat-treated and pasteurized milk [11,31] even more frequently than in those made from unpasteurized [20], which is probably due to a post-treatment contamination [36].

The ubiquity of *L. monocytogenes* in nature and its recognized presence in food-processing environments [49] explain the difficulty in producing either minimally processed foods or ones handled after processing that are free of the pathogen.

A relatively small number of cases of foodborne diseases are caused by *L. monocytogenes* in comparison to the most common pathogen outbreaks such as those caused by *Salmonella* spp. or *Campylobacter jejuni* [12, 21]. Despite its low incidence, the infection is considered of great impor-

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tance due to the severity of the illness and high mortality rates (e.g., 12.70% in 2011 vs. 0.12% for *Salmonella*) [21].

Recently, the effectiveness of accelerated electrons (E-beam) treatment to eliminate pathogens was demonstrated [8,10,28,39] in a variety of ready-to-eat (RTE) foods. Moreover, a higher radioresistance of *L. innocua* vs. *L. monocytogenes* has been consistently observed [8,10,28,42] permitting the former species to be used as a surrogate of *L. monocytogenes*.

Previously, we have used E-beam radiation [52] to minimize the incidence of late blowing of cheese but an important reduction in lactic acid bacteria (LAB) was also produced, although after damage reparation an almost normal growth was recorded.

Taking these findings into account, it was postulated that E-beam technology could be highly appropriate for sanitizing RTE cheeses. This paper describes a study to optimize E-beam treatment of the above mentioned cheese varieties with the final goal of reducing the number of *L. monocytogenes* to a safe level, i.e., in compliance with the criteria of EU and USDA regulations for this bacterium.

Materials and methods

Organisms. One strain of *Listeria innocua* (NCTC 11288) and another of *L. monocytogenes* (Scott A, CIP 103575, serotype 4b) were used. The strains were maintained by freezing (-40°C) in trypticase soy broth (TSB; Difco, Detroit, MI, USA) with 10% glycerol added as a cryogenic agent. Fresh cultures were prepared by removing a piece of frozen culture from vials and inoculating it into 9 ml of TSB, then incubating it at 32°C for 24 h. The culture was then centrifuged at 4°C ($3000 \times g$ for 30 minutes) and the pellet was suspended in a beaker with 50 ml sterile saline solution, which yielded a bacterial load of approximately 10^8 cells/ml. In experiments, large numbers of cells were used to accurately determine the bacterium inactivation kinetics.

Sample preparation and irradiation treatment. Individually wrapped portions (30 g) of Brie and Camembert cheeses made from pasteurized milk were acquired at a local supermarket. Only samples used to study the effect of E-beam on the survival of both *L. monocytogenes* and *L. innocua* were deliberately contaminated. To do this, 0.5 ml of the bacterial suspension was divided in five aliquots of 0.1 ml, which were injected with disposable syringes in five separated zones of the cheese portion after removing wrappings and rinds aseptically. Then, samples were vacuum-packaged in 20×20 cm laminated film bags of low gas permeability (oxygen transmission rate of $35 \text{ cm}^3/24 \text{ h m}^2 \text{ bar}$ and $150 \text{ cm}^3/24 \text{ h m}^2 \text{ bar}$ to carbon dioxide) until vacuum reached 20 kPa. Once the samples were ready, they were treated in the irradiation plant (Ionisos Iberica, SA, Tarancón, Spain) and irradiated under an electron beam radiation source, which operates at 10 MeV. The radiation doses employed were between 0.2 and 2 kGy for kinetics studies and from 1 to 3 kGy for texture and sensory analyses. The dose absorbed by samples was checked by determining the absorbance of cellulose triacetate dosimeters [1] simultaneously irradiated with samples. Experiments were made in triplicate and performed at room temperature ($18\text{--}20^{\circ}\text{C}$). The product temperature increased less than 2°C during treatment. Following the treatment, samples

were transferred to the laboratory where samples intended for determining the death kinetics and the selected parameters corresponding to the day zero were immediately processed. The remaining samples were stored at 4 and 14°C (the latter as an example of temperature abuse) to study the microbiota and behaviour of selected characteristics over time.

Physicochemical analysis. Moisture, ash, protein, fat, pH and *aw* were measured as previously described [52]. The pH was determined in the rind and core of cheese samples, using a Crison Digit-501 pH meter (Crison Instruments LTD, Barcelona, Spain).

Microbial analyses. To count survivors, the samples, after weighing, were homogenized with 20 ml of sterile saline solution in a Stomacher bag. Total viable counts (TVC) were determined by the pour-plate method using Plate Count Agar (PCA; Difco) as culture medium. Lactobacilli counts were performed in double layer acidified (pH 5.5) MRS agar (Conda-Pronadisa, Madrid, Spain) as recommended by Henri-Dubernet et al. [26]. Incubation was carried out at 32°C for 48 h.

To determine inactivation parameters of both *L. monocytogenes* and *L. innocua*, Palcam agar base (Oxoid, Basingstoke, UK) with egg yolk emulsion (Oxoid) and selective supplement (polymyxin B, acriflavine HCl and ceftazimide, Oxoid) was used. Plates were incubated at 37°C for 48 h. Despite the selective agents present in the Palcam agar, this medium has been found previously to be perfectly suitable for the determination of listeria death kinetics [9]. Colonies were enumerated with a Digital S Colony counter (J. P. Selecta, Barcelona, Spain).

Survival curves were constructed by plotting log CFU/g against irradiation dose. Decimal reduction doses (*D*-values) were calculated from the linear regression equation of survival curves. The TVC and LAB growth curves were constructed according to the Baranyi and Roberts model [4] using the Excel add-in fitting curves Dmfit [<http://www.ifr.ac.uk/safety/dmfit/>].

Risk assessment. For risk assessment the USDA recommends a “zero tolerance” policy for *L. monocytogenes* in RTE products, equivalent to a Food Safety Objective (FSO) of 4 CFU/100g ($\log_{10} = -1.39$). In the EU, European Commission Regulation (EC) No. 1441/2007 [18] divides the RTE foods into two categories according to the ability of *L. monocytogenes* to grow in them. So, for the material with an a_w above 0.92, which permits the growth of *L. monocytogenes*, the “zero tolerance” criterion would be applied. Products must not contain *L. monocytogenes* in 25 g at the time they leave the production plant. When the product is on the market, the limit is 100 CFU/g ($\log_{10} = 2$) throughout the shelf life [18].

To establish the Process Criteria (PC), i.e., the irradiation dose required to reach the FSO, the initial contamination and the listeria growth throughout the shelf-life must be taken into account. The current milk pasteurization conditions are sufficient to achieve a decrease of 5-6 decimal reductions in *L. monocytogenes* [16]. In cheeses made from pasteurized milk, the original listeria level therefore depends upon the potential contamination during the preparation of portions.

The post-milking environment has been identified as the main source of contamination with *L. monocytogenes*. This was detected in milk from 90% of herds although at low concentrations, accounting for around 2.25 CFU/ml in bulk tank milk [5], but pasteurization causes a reduction of 5.2 log units of *L. monocytogenes* according to the data reported by Mackey and Bratchell [37], resulting in a listeria concentration of 1.42×10^{-5} cells/ml. To manufacture each piece of Camembert (250 g) and Brie cheese (2,250 g), 2.2 and 22.5 litres of milk are required, respectively [45]. During whey draining, 10% of bacterial cells should be eliminated [45]. So, 0.03 and 0.29 cells should be retained in each Camembert and Brie curd, respectively (ca. 10^{-4} cell/g). Ryser and Marth [44] analysed the changes in *L. monocytogenes* numbers during the manufacture and ripening of Camembert cheeses made from raw milk artificially contaminated with 2.6–2.9 log CFU/ml. After 65 days of ripening,

counts reached 6.30–7.51 log CFU/g. A similar increase was obtained in the listeriosis risk analysis carried out by Sanaa et al. [45] for other white mould ripened cheeses. From an initial load of 0.8 and 0.3 cells of *L. monocytogenes* per litre of raw milk, the former authors calculated, at the time of consumption, 3 and 5 cells/g in Camembert and Brie varieties, respectively. Therefore, if the estimated increase is about 4 log units, from an initial concentration in the curd of 1.12×10^{-4} and 1.29×10^{-4} cell/g (i.e., 0.03 and 0.29 cells/curd) the Camembert and Brie cheeses should contain a final load of 1.12 and 1.28 CFU/g ($\log_{10} = 0.05$ – 0.11). These values are lower compared to the contamination that could occur post-processing or during size reduction adopted by other authors for products such as sausages [29] and cooked ham [8], assumed to be around 10 cells/g ($\log_{10} = 1$). Following the same criterion for the preparation of cheese portions, *L. monocytogenes* contamination during this operation could reach, in the worst case, the amount of 10 cells/g. On the other hand, from the results of several authors compiled by FDA [22], a growth rate of 0.071 log CFU/day for *L. monocytogenes* in Camembert and Brie cheeses stored at 4°C has been calculated. Assuming for cheeses, a shelf-life of 60 days at 4°C, a final bacterial load of 5.26 log units ($0.071 \times 60 + 1$) would be achieved. From these data, it can be estimated that 3.26 and 6.65 decimal reductions would be required (performance criteria) to reach the FSO for EU and USDA statements, respectively.

Texture analysis. Cheeses were sampled on day 0 and after 15 (two weeks) and 42 days (six weeks) of storage. The effect of E-beam radiation on the texture of cheeses and how it changes during storage were examined through a puncture test, as described by Herrero et al. [27], and a texture profile analysis (TPA) as previously reported [52]. Hardness, springiness, adhesiveness, cohesiveness, gumminess and chewiness parameters of samples were calculated.

Sensory analysis. A triangular test, performed as described by Velasco et al. [52], was carried out at different days during storage at both temperatures to study the behaviour of appearance, flavour and odour of samples E-beam treated. In addition, panellists were asked to justify their answer with brief descriptions.

Statistical analysis. Excel (Microsoft, Redmond, WA, USA) was used to calculate the coefficients of determination (R^2) of survival curves and to conduct the F test to compare them. For statistical analysis of the physicochemical tests results, a one-way ANOVA and Duncan's test for multiple-range test were performed using Statgraphics Centurion XVI for Windows (Statistical Graphics Corporation, Rockville, MD, USA).

Results and Discussion

Physicochemical characteristics. The chemical composition (% on wet matter) of Camembert and Brie cheeses was: moisture, 45.08 ± 1.09 and 47.13 ± 0.85 ; fat, 31.90 ± 0.14 and 36.77 ± 3.38 ; protein 24.52 ± 1.56 and 18.98 ± 0.65 ; and ash 2.88 ± 0.11 and 2.66 ± 0.21 , respectively. Moisture values were slightly lower than those reported in the literature (45–47% vs. 48–52%) while the fat (32–37% vs. 24–27%) and protein (19–24% vs. 19–21%) contents were higher [13,23], but, consequently, both compounds were similar in dry matter terms.

No significant ($P > 0.05$) dose effect was observed in the a_w values immediately after irradiation treatment in both

cheeses (Table 1), as previously recorded in cheese slices [52]. The initial values were 0.960 and 0.964 for Camembert and Brie, respectively (Table 1), similar to values (0.967 and 0.965) found by Marcos et al. [38] for both cheeses and slightly lower than the value (0.97) reported by Guinee and Fox [25]. At the end of storage, the higher the dose the higher the a_w of Brie samples, which reached values of 0.983 and 0.974 in samples treated at 3 kGy and stored for 41 days at 4°C and 19 days at 14°C, respectively with some significant differences ($P < 0.05$). However, this trend was not observed in the Camembert cheese because there was a significant increase ($P < 0.05$) in this parameter during storage, independently of the dose applied. After 41 days storage at 4°C and 19 days at 14°C, the average values were 0.978 and 0.973, respectively. The radiolysis of water present in both the cheese and the package environment resulted in the release of hydroxyl radicals, whose concentration diminished over time to return to form water molecules [46] and could contribute to an increase in water activity at the end of storage. Nevertheless, although in some instances significant differences were found, the range of values, from 0.983 and 0.957 (Table 1), was not broad enough to considerably inhibit either the growth of *L. monocytogenes* or the dominant microbiota (LAB), since the minimum a_w for growth of the former pathogen has been established at 0.92 [16] and LAB may grow perfectly well at a_w below 0.96 [50]. Indeed, the drop in a_w is the basis of the lactic fermentation that occurs in traditional dry fermented sausages by LAB [43].

In the core of both cheeses, no relation between storage time and pH values was observed (data not shown). No dose effect ($P > 0.05$) was found in the pH at any of the times tested, consistent with the findings reported by various authors for irradiated cheeses, e.g., Konteles et al. [34]. Figure 1 shows the effect of E-beam treatment on the pH values in rind of Camembert and Brie cheeses and their changes during storage at 4 and 14°C. At the beginning of storage, the rinds had an average pH of 7.21 (Camembert) and 6.90 for (Brie), whereas inside the pH was significantly ($P < 0.05$) lower, with values of ca. 5.95 and 6.29. These values are in agreement with those reported by Spinnler and Gripon [48]. The increasing pH gradient from the centre to the surface is characteristic of white mould-ripened cheeses since *Penicillium camemberti*, present on the cheese surface, uses lactate as a carbon source for cell growth [35], which would result in a deacidification of the surface of the cheese. *Geotrichum candidum* might be implicated in the ripening of Camembert-type cheese [48], also helping to raise the pH either by the metabolism of amino acids as carbon and nitrogen sources

Table 1. Effect of E-beam treatment and storage conditions on a_w (mean values \pm SD) of Camembert and Brie cheeses

Cheese	Storage		Dose			
	(°C—days)		0 kGy	1 kGy	2 kGy	3 kGy
Camembert	4	0	0.960 \pm 0.002 ^b	0.957 \pm 0.002 ^c	0.964 \pm 0.005 ^b	0.962 \pm 0.006 ^b
		26	0.957 \pm 0.002 ^{b,b}	0.966 \pm 0.002 ^{a,b}	0.967 \pm 0.002 ^{a,b}	0.969 \pm 0.003 ^{a,b}
		41	0.977 \pm 0.003 ^a	0.979 \pm 0.001 ^a	0.976 \pm 0.003 ^a	0.981 \pm 0.005 ^a
	14	0	0.960 \pm 0.002 ^b	0.957 \pm 0.002 ^b	0.964 \pm 0.005	0.962 \pm 0.006
		19	0.975 \pm 0.003 ^{a,a}	0.972 \pm 0.003 ^{a,β,a}	0.969 \pm 0.002 ^{β}	0.972 \pm 0.003 ^{a,β}
	Brie	4	0	0.964 \pm 0.009	0.958 \pm 0.001 ^f	0.974 \pm 0.007
27			0.966 \pm 0.007	0.969 \pm 0.001 ^e	0.969 \pm 0.009	0.970 \pm 0.001 ^e
41			0.966 \pm 0.003 ^{γ}	0.976 \pm 0.004 ^{β,d}	0.978 \pm 0.003 ^{a,β}	0.983 \pm 0.001 ^{a,d}
14		0	0.964 \pm 0.009	0.958 \pm 0.001	0.974 \pm 0.007	0.965 \pm 0.011
		19	0.957 \pm 0.001 ^{β}	0.959 \pm 0.002 ^{β}	0.971 \pm 0.002 ^a	0.974 \pm 0.004 ^a

^{a, β , γ} : values in the same row with a different letter are significantly different ($P < 0.05$).

^{a, b, c} and ^{d, e, f}: values in the same column with a different letter are significantly different in Camembert and Brie cheese, respectively ($P < 0.05$).

yielding ammonia or lactate utilization to maintain the cell structure during the stationary phase [3]. Furthermore, *Debaryomyces hansenii*, a yeast not included in the starters but possibly present because of their ubiquitous nature, could contribute to the surface alkalisation [35]. During storage, the pH of the rinds became significantly more acid ($P < 0.05$) in all the samples until reaching a value of around 6.0 for both Camembert and Brie cheeses after 41 days of storage at 4°C and 5.67 and 5.84, respectively, after 19 days of storage at 14°C. This phenomenon could be explained by both the facultative anaerobic nature of LAB and the progressive death of the moulds caused by the low level of oxygen in the package, supported by the progressive disappearance of mycelium.

Microbial results. *Lactococcus* [13] and lactobacilli [26] are the dominant organisms in the paste of Camembert and Brie cheeses. Then, the fate of LAB could be assimilated to that of the TVC. The response of LAB microbiota (PCA counts) and lactobacilli (MRS counts) to the action of E-beam treatment is shown in Fig. 2. Initially, the TVC accounted was about 8.0–8.5 log CFU/g in both cheeses, which is normal for most cheese varieties [24]. The 1 kGy treatment resulted in a

decrease of about 2 (Camembert) and 3 (Brie) log units. When 2 kGy were applied, the reductions were around 5 and 4 log units, respectively. At 3 kGy a “tail” was observed in both cheeses, which may be explained by the presence of a heterogeneous indigenous microbiota consisting of the most radioresistant organisms, which would be responsible for the tail. These organisms would belong to bacterial groups other than LAB because at 3 kGy, the lactobacilli (Fig. 2) had practically disappeared since the values came from counts of 3–4 colonies developed on the agar plates. Although lactobacilli counts of 3 kGy samples were approximated, they were fitted to a straight line, which confirms the well-known first order kinetics of bacteria inactivation by ionizing radiation. Since the straight portions of survivor curves were parallel, global D -values of 0.42 and 0.51 kGy could be roughly estimated for Camembert and Brie cheeses, respectively. These results are close to the range recorded by other authors. For example, a D -value of 0.66 kGy has been reported for mesophilic aerobic count in cheese slices [52] and 0.39 kGy could be estimated for TVC in Cottage cheese [32]. The differences may be explained by the fact that cheese always presents a mixed microbiota and the radioresistance of bacteria may be variable [52]. However, in some cheese varieties, much higher D -values have been re-

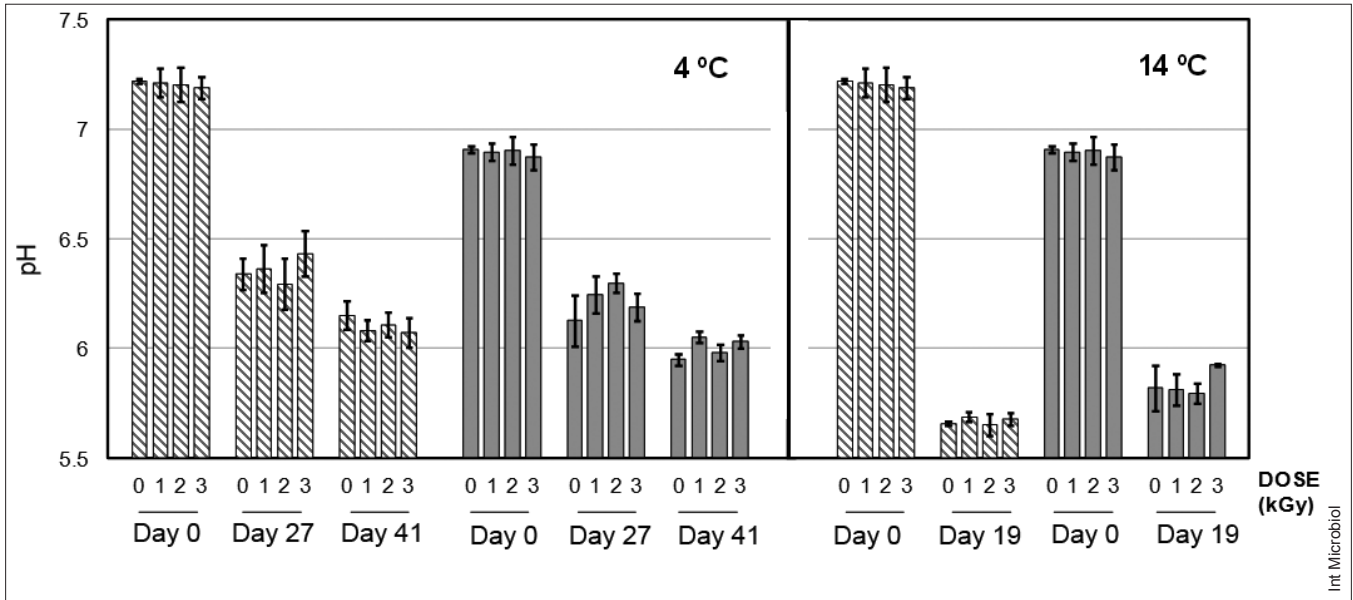


Fig. 1. Changes in the rind pH during storage at 4 and 14°C of Camembert (striped bars) and Brie (solid bars) cheeses treated by E-beam irradiation.

ported, such as 1.8 kGy in Feta cheese [34], probably due to factors related to bacterial radioresistance, which depend on both the inherent resistance of the organisms and the conditions of the matrix food [2,6].

The changes in TVC (total LAB) in Camembert cheese during storage at 4 and 14°C after E-beam treatment are shown in Fig. 3. As expected, no changes occurred in control samples throughout storage. In samples stored at 14°C, a quick micro-

biota recovery was observed when 1 kGy was applied in such a way that in a couple of days the number of TVC superposed that of control (non-treated) samples, growing at a doubling time (*g* value) of 12 h. The same pattern was detected at 2-kGy doses but the growth rate was slower (*g* of about 24 h) achieving the 10^8 CFU/g level after 12 days of storage. The bacteria subjected to 3 kGy were probably not able to completely repair the damage produced by the E-beam and the highest level of

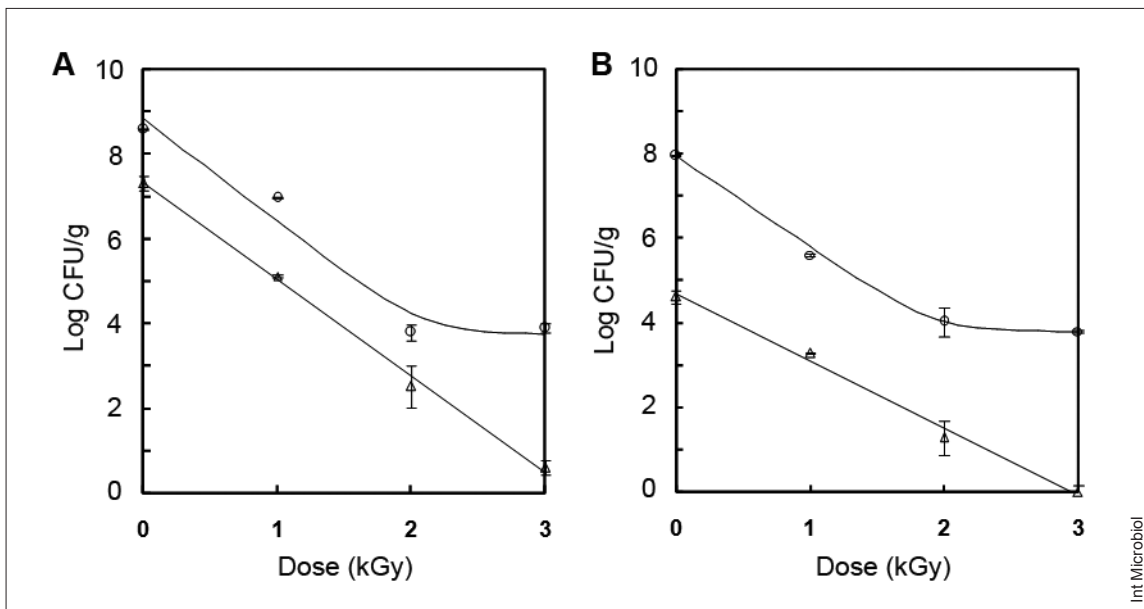


Fig. 2. Survival curves of LAB (circles) and lactobacilli (triangles) for Camembert (A) and Brie (B) rindless cheeses.

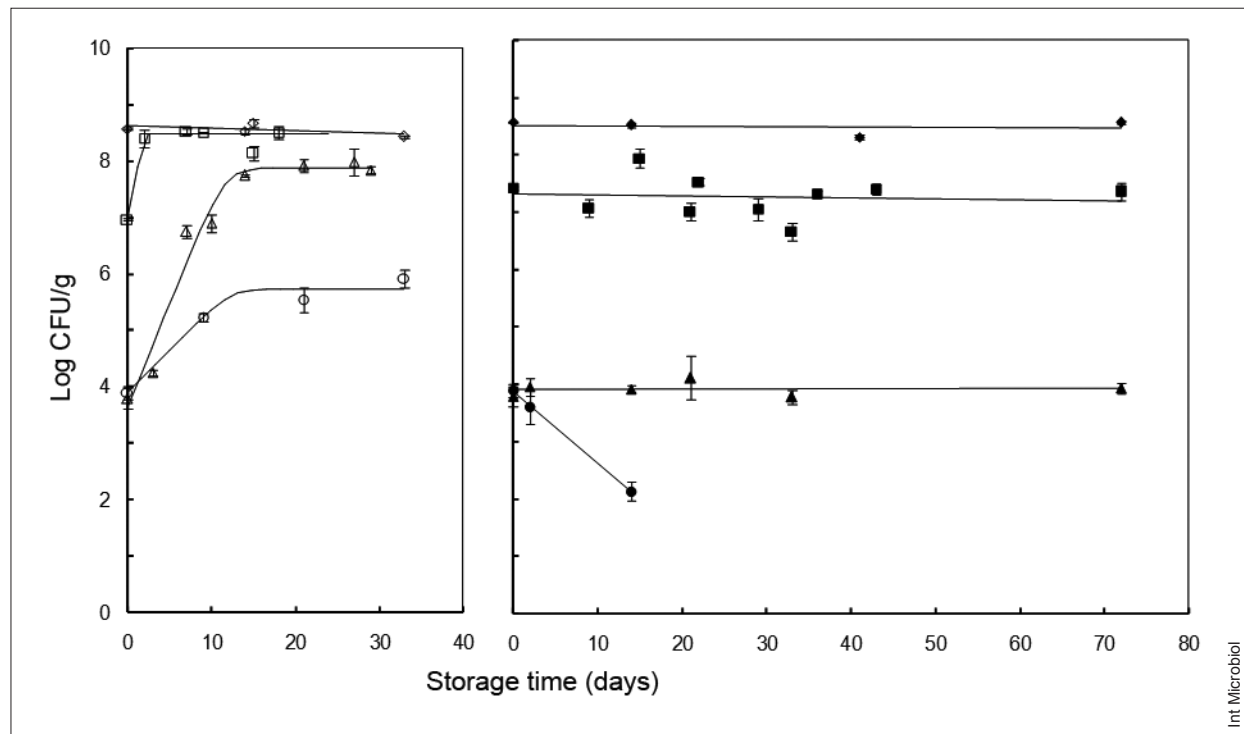


Fig. 3. TVC of control (diamonds) and irradiated Camembert cheese at 1 (squares), 2 (triangles) and 3 kGy (circles) stored at 4°C (full symbols) and 14°C (empty symbols).

them did not exceed 10^6 CFU/g after 33 days of storage. At 4°C, the TVC was maintained (at doses of 1 and 2 kGy) at the levels achieved just after the treatment application, but at 3 kGy a decrease was observed afterwards and no colonies were detected after 20 days of storage. TVC changes in the Brie cheese showed a similar trend than in the Camembert (data not shown). Briefly, the revival of microbiota was observed estimating a lag phase of about 10 days at 14°C when doses of 2 and 3 kGy were used and at 4°C even a slight growth occurred when 1 kGy was applied, giving a g value of about 500 h. In the lactobacilli counts, the same pattern was observed in both Camembert and Brie cheeses (data not shown). From all the former data taken together, it would seem that, although the LAB are the most important bacteria in both cheeses, the species present in the Brie cheese used in this work were more

sensitive than those of the Camembert variety. In a previous study on the use of E-beam radiation to diminish the late blowing of cheese, a recovery of the total mesophilic aerobic counts was reported, although the profile observed was slightly different [52].

Food safety aspects. According to the EU regulation [18], both cheeses are included in the category of RTE foods able to support the growth of *L. monocytogenes* since both the pH and a_w are higher than 4.4 and 0.92, respectively. It is, therefore, a risk for the consumer if this bacterium is present. The response of both *L. innocua* and *L. monocytogenes* to the E-beam treatment resulted in a first-order inactivation kinetics, as repeatedly reported [e.g. 39,40]. From the regression equations shown in Table 2, D -values 0.39 kGy for *L. innocua* and

Table 2. Irradiation decimal reduction values (D -values) for *Listeria monocytogenes* and *Listeria innocua* in Camembert and Brie cheeses

Cheese	Microorganism	Survival equations	R^2	D -value
Camembert	<i>L. innocua</i> NCTC 11288	$\text{Log CFU/g} = 7.42 - 2.80 \cdot \text{Dose}$	0.96	0.36 kGy
	<i>L. monocytogenes</i> Scott A	$\text{Log CFU/g} = 9.26 - 2.95 \cdot \text{Dose}$	0.995	0.34 kGy
Brie	<i>L. innocua</i> NCTC 11288	$\text{Log CFU/g} = 7.29 - 2.55 \cdot \text{Dose}$	0.94	0.39 kGy
	<i>L. monocytogenes</i> Scott A	$\text{Log CFU/g} = 9.26 - 2.92 \cdot \text{Dose}$	0.999	0.34 kGy

0.34 kGy for *L. monocytogenes* can be calculated in Brie cheese and 0.36 and 0.34 kGy, respectively, in Camembert cheese. *D*-values of a similar order have been reported for *L. monocytogenes* in processed cheese slices treated with gamma radiation [47]. Higher *D*-values have been reported at refrigeration temperatures [32,33,50]: 0.84–0.93 kGy in sliced and pizza cheeses (10°C), 1.33 kGy in Feta cheese (0–2°C), and 1.38 kGy in soft whey cheese Anthotyros (4°C), respectively.

To estimate the dose required to meet the FSO (process criteria), the most unfavourable case was taken into account. Thus, the highest *D*-value, matching *L. innocua* in Brie cheese, was chosen (0.39 kGy). According to the previously calculated performance criterion, 3.26 and 6.65 decimal reductions would be required depending on the destination country. Therefore, the process criteria would be 1.27 and 2.59 kGy, respectively. These doses are much lower (the former) and of the similar level (the latter) than that considered as acceptable (i.e., 2.5 kGy) by the Scientific Committee on Food of EU and allowed in France for the treatment of Camembert cheese made from raw milk [19]. However, Bougle and Stahl [7] have detected viable listeria but they were not able to grow at 12°C in Camembert cheese treated at 2.6 kGy. Likewise, a slower growth rate of *L. monocytogenes* and *S. aureus* has been observed in vacuum packaged cooked ham after treatment at 2 and 3 kGy [9].

Texture and sensory aspects. Immediately after E-beam treatment, no significant differences ($P > 0.05$) in some textural attributes (adhesiveness, springiness and chewiness) were found in both cheeses. However, significant differences ($P < 0.05$) in other parameters (hardness, cohesiveness, gumminess and breaking force) were detected only in Camembert cheese while values for the Brie samples remained fairly constant. Nevertheless, no significant differences ($P > 0.05$) between untreated and treated samples after two weeks of storage were detected. A similar behaviour was observed in sensorial parameters. These results are in agreement with those by other authors, e.g., no significant differences were found in the sensory attributes of Camembert samples treated with doses up to 2.5 kGy [14]. Similarly, it has been reported that the texture of Feta and smear-ripened cheeses is not affected by irradiation treatments [17,50].

From the results obtained, it can be concluded that E-beam treatments at 1.27 and 2.59 kGy allowed the control of *L. monocytogenes* growth, achieving the FSO in soft mould-ripened cheeses (Camembert and Brie varieties) according to EU and USDA criteria, respectively. Although the lactic acid microbiota was also reduced, its recovery occurred afterwards

in samples stored at 14°C (a favourable temperature for LAB growth) until reaching values close to the initial numbers.

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Competing interests. None declared.

References

1. American Society for Testing and Materials (2000) E1650-97e1 standard practice for use of cellulose acetate dosimetry systems. 12:2. ASTM, West Conshohocken, PA, USA
2. Augustin JC (1996) Resistance of *Listeria monocytogenes* to physical exposure. Pathol Biol 44:790-807
3. Aziza M, Adour L, Amran, A (2008) Assimilation of peptides and amino acids and dissimilation of lactate during submerged pure cultures of *Penicillium camembertii* and *Geotrichum candidum*. J Microbiol Biotechnol 18:124-127
4. Baranyi J, Roberts, TA (1994) A dynamic approach to predicting bacterial growth in food. Int J Food Microbiol 23:277-294
5. Bemrah N, Sanaa M, Cassin MH, Griffiths MW, Cerf O (1998) Quantitative risk assessment of human listeriosis from consumption of soft cheese made from raw milk. Prev Vet Med 37:129-145
6. Black JL, Jaczynski J (2008) Effect of water activity on the inactivation kinetics of *Escherichia coli* O157:H7 by electron beam in ground beef, chicken breast meat, and trout fillets. Int J Food Sci Tech 43:579–586
7. Bougle DL, Stahl V (1994) Survival of *Listeria monocytogenes* after irradiation treatment of Camembert cheeses made from raw milk. J Food Protect 57: 811-813
8. Cabeza MC, Cambero I, de la Hoz L, Ordóñez JA (2007) Optimization of E-beam irradiation treatment to eliminate *Listeria monocytogenes* from ready-to-eat (RTE) cooked ham. Innov Food Sci Emerg 8:299-305
9. Cabeza MC, Cambero MI, Núñez M, Medina M, de la Hoz L, Ordóñez J (2010) Lack of growth of *Listeria monocytogenes* and *Staphylococcus aureus* in temperature abuse of E-beam treated ready-to-eat (RTE) cooked ham. Food Microbiol 27:777-782
10. Cambero MI, Cabeza MC, Escudero R, Manzano S, García-Márquez I, Velasco R, Ordóñez JA (2012) Sanitation of selected ready-to-eat (RTE) intermediate moisture foods (IMF) of animal origin by E-beam irradiation. Foodborne Pathog Dis 9:594-599
11. Centers for Disease Control and Prevention (2013) Multistate outbreak of listeriosis linked to Crave Brothers Farmstead cheeses. Available at: <http://www.cdc.gov/listeria/outbreaks/cheese-07-13/index.html>.
12. Centers for Disease Control and Prevention (2013) Summary of notifiable diseases—United States 2011. Morb Mortal Wkly Rep 60:1-117
13. Chapman HR, Sharpe ME (1990) Microbiology of cheese. In Robinson RK (ed) Dairy microbiology, vol. 2 The microbiology of milk products. 2nd ed. Elsevier, Essex, UK, pp 203-289
14. Chincholle R (1991) Action of the ionization treatment on the soft cheese made from unpasteurized milk. C R Acad Agric Fr 77:29-35
15. Codex Alimentarius Commission (2009) Guidelines on the application of general principles of food hygiene to the control of *Listeria monocytogenes* in Ready-To-Eat foods. CAC/GL 61-2007, Rev. 1. Available at: http://www.codexalimentarius.org/download/standards/10740/CXG_061e.pdf. Accessed 9 January 2015

16. Doyle ME, Mazzota AS, Wang T, Wiseman DW, Scott VN (2001) Heat resistance of *Listeria monocytogenes*. J Food Protect 64:410-429
17. Ennahar S, Kuntz F, Strasser A, Bergaentzle M, Hasselmann C, Stahl V (1994) Elimination of *Listeria monocytogenes* in soft and red smear cheeses by irradiation with low-energy electrons. Int J Food Sci Tech 29:395-403
18. European Commission (2007) Commission Regulation (EC) No 1441/2007 of 5 December 2007 amending Regulation No 2073/2005 on microbiological criteria for foodstuffs. Off J Eur Union L322:12-29
19. European Food Safety Authority (2011) Statement summarizing the conclusions and recommendations from the opinions on the safety of irradiation of food adopted by the BIOHAZ and CEF Panels. EFSA J 9: 2107
20. European Food Safety Authority (2011) The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2009. EFSA J 9:2090
21. European Food Safety Authority (2013) The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2011. EFSA J 11:3129
22. US Food and Drug Administration, US Food Safety and Inspection Service (2003) Quantitative assessment of relative risk to public health from foodborne *Listeria monocytogenes* among selected categories of ready-to-eat foods. Appendix 8: Growth of *Listeria monocytogenes* in foods. Available at: <http://www.fda.gov/downloads/Food/FoodScienceResearch/UCM197321.pdf>
23. Food Standards Agency (2002) McCance and Widdowson's the composition of foods, 6th ed. Royal Society of Chemistry, Cambridge, UK
24. Fox PF, McSweeney PLH, Cogan TM, Guinee TP (2004) Cheese: Chemistry, physics and microbiology, Vol. 2 Major Cheese Groups. 3rd ed. Elsevier Applied Science, London, UK
25. Guinee TP, Fox PF (2004) Salt in cheese: Physical, chemical and biological aspects. In Fox PF, McSweeney PLH, Cogan TM, Guinee TP (eds) Cheese: Chemistry, physics and microbiology, Vol. 2 Major Cheese Groups, 3rd ed. Elsevier Applied Science, London, UK, pp 208-259
26. Henri-Dubernet S, Desmasures N, Guéguen M (2008) Diversity and dynamics of lactobacilli populations during ripening of RDO Camembert cheese. Can J Microbiol 54:218-228
27. Herrero AM, Cambero MI, Ordóñez JA, de la Hoz L, Carmona P (2009) Plasma powder as cold-set binding agent for meat system: Rheological and Raman spectroscopy study. Food Chem 113:493-499
28. Hoz L, Cambero MI, Cabeza MC, Herrero AM, Ordóñez JA (2008) Elimination of *Listeria monocytogenes* from vacuum-packed dry-cured ham by E-Beam radiation. J Food Protect 71:2001-2006
29. International Commission on Microbiological Specifications for Foods (2002) *Listeria monocytogenes* in cooked sausage (Frankfurters). In Tompkin RB, Garam L, Roberts TA, Buchanan RL, van Schothorst M, Dahms S, Cole MB (eds), Microorganisms in foods: Microbiological testing in food safety management, vol. 7. Kluwer/Plenum, New York, USA, pp 285-312
30. Jackson KA, Biggerstaff M, Tobin-D'Angelo M, Sweat D, Klos R, Nosari J, Garrison O, Boothe E, et al. (2011) Multistate outbreak of *Listeria monocytogenes* associated with Mexican-style cheese made from pasteurized milk among pregnant, Hispanic women. J Food Protect 76:949-953
31. Johnson EA, Nelson JH, Johnson M (1990) Microbiological safety of cheese made from heat-treated milk, Part I. Executive summary, introduction and history. J Food Protect 53:441-452
32. Jones TH, Jelen P (1988) Low dose-irradiation of Camembert, Cottage cheese and Cottage whey. Milchwissenschaft 43:233-235
33. Kim HJ, Ham JS, Lee JW, Kim K, Ha SD, Jo C (2010) Effects of gamma and electron beam irradiation on the survival of pathogens inoculated into sliced and pizza cheeses. Radiat Phys Chem 79:731-734
34. Konteles S, Sinanoglou VJ, Batrinou A, Sflomos K (2009) Effects of γ -irradiation on *Listeria monocytogenes* population, colour, texture and sensory properties of Feta cheese during cold storage. Food Microbiol 26:157-165
35. Lessard MH, Bélanger G, St-Gelais D, Labrie S (2012) The composition of Camembert cheese ripening cultures modulates both mycelial growth and appearance. Appl Environ Microb 78:1813-1819
36. Lianou A, Sofos JN (2007) A review of the incidence and transmission of *Listeria monocytogenes* in ready-to-eat products in retail and food service environments. J Food Protect 70:2172-2198
37. Mackey BM, Bratchell N (1989) The heat resistance of *Listeria monocytogenes*: A review. Lett Appl Microbiol 9:89-94
35. Marcos A, Esteban MA, Alcalá M (1990) Determination of water activity in Brie and Camembert cheese varieties by four different methods. Food Chem 38:189-199
39. Medina M, Cabeza MC, Bravo D, Cambero I, Montiel R, Ordóñez JA, Nuñez M, Hoz L (2009) A comparison between E-beam irradiation and high pressure treatment for cold-smoked salmon sanitation: microbiological aspects. Food Microbiol 26:224-227
40. Miller RB (2005) Electronic irradiation of foods: An introduction to the technology. Springer Science, New York, USA
41. Moseley BEB (1989) Ionizing radiation: Action and repair. In Gould GA (ed.), Mechanisms of action of food preservation procedures. Elsevier, Essex, UK, pp 43-70
42. Niemira BA, Fan X, Sokorai KJB, Sommers CH (2003) Ionizing radiation sensitivity of *Listeria monocytogenes* ATCC 49594 and *Listeria innocua* ATCC 51742 inoculated on endive (*Cichorium endiva*). J Food Protect 66:993-998
43. Ordóñez JA, Hierro EM, Bruna JM, de la Hoz L (1999) Changes in the components of dry-fermented sausages during ripening. Crit Rev Food Sci 39:329-367
44. Ryser ET, Marth EH (1987) Fate of *Listeria monocytogenes* during manufacture and ripening of Camembert cheese. J Food Protect 50:372-378
45. Sanaa M, Coroller L, Cerf O (2004) Risk assessment of listeriosis linked to the consumption of two Soft cheeses made from raw milk: Camembert of Normandy and Brie of Meaux. Risk Anal 24:389-399
46. Seisa D, Osthoff G, Hugo C, Hugo A, Bothma C, Van der Merwe J (2004) The effect of low-dose gamma irradiation and temperature on the microbiological and chemical changes during ripening of Cheddar cheese. Radiat Phys Chem 69:419-431
47. Sommers CH, Boyd G (2005) Elimination of *Listeria monocytogenes* from Ready-to-Eat turkey and cheese tortilla wraps using ionizing radiation. J Food Protect 68:164-167
48. Spinnler HE, Gripon JC (2004) Surface mould-ripened cheeses. In Fox PF, McSweeney PLH, Cogan TM, Guinee TP (eds) Cheese: Chemistry, physics and microbiology, Vol. 2 Major Cheese Groups, 3rd ed. Elsevier Applied Science, London, UK, pp 208-259
49. Taormina PJ, Beuchat LR (2001) Survival and heat resistance of *Listeria monocytogenes* after exposure to alkali and chlorine. Appl Environ Microb 67:2555-2563
50. Troller JA, Stinson JV (1981) Moisture requirements for growth and metabolite production by Lactic Acid Bacteria. Appl Environ Microb 42:682-687
51. Tsiotsias A, Savvaidis I, Vassila A, Kontominas M, Kotzekidou P (2002) Control of *Listeria monocytogenes* by low-dose irradiation in combination with refrigeration in the soft whey cheese 'Anthotyros'. Food Microbiol 19:117-126
52. Velasco R., Ordóñez JA, Cabeza MC, Hoz L, Cambero MI (2011) Use of the E-beam radiation to diminish the late blowing of cheese. Int Dairy J 21:493-500
53. Warriner K, Namvar A (2009) What is the hysteria with *Listeria*? Trends Food Sci Tech 20:245-254