Article

Application of Gamma Irradiation Treatment on the Physicochemical and Microbiological Quality of an Artisanal Hard Cheese

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Featured Application: Small-scale cheese producers can effectively use the irradiation treatment for eliminating food-borne pathogens.

Abstract: The objective of this study was to evaluate the efficacy of gamma irradiation, applied to different cheese sample sizes (250g and 500 g), against *Listeria monocytogenes, Escherichia coli,* coliforms and aerobic colony counts. The effects on cheese physicochemical and odour properties and all costs involved for the treatment were quantified. The Cobalt-60 γ-irradiator was used at a maximum dose of 5.0 kGy. The values for cheese moisture (28.6%), ash (3.78%), pH (5.1), protein (29.6%), fat (30.7%), salt (1.95%) and water activity (0.92%) were within the acceptable ranges for hard cheese after gamma irradiation treatment. The colour (yellowness, redness, chroma and hue angle) and texture (cohesiveness and springiness) values decreased ($p < 0.05$) with the treatment. Compounds such as safrole, acetylpyrazine, thiophene, 3,5-octadien-2-one and 1-Octen-3-one were present after the treatment, regardless of sample size. The gamma irradiation treatment resulted in 100%, 87.2%, 85.1% and 77.3% reduction in *L. monocytogenes*, coliforms, *E. coli* and aerobic colony counts, respectively. The study highlighted the efficacy of irradiation treatment and its affordability for resource-limited producers.

Keywords: cheese; irradiation cost; ionising radiation; microbial safety; Smart nose; gas chromatography olfactometry

1. Introduction

Several treatments have been adopted with the aim of improving the microbial safety of cheese, both during and after processing. Milk pasteurisation is used to reduce microbial counts in milk before cheese-making, but this is not an absolute safeguard, since there can still be chances of post-pasteurisation contamination [1,2]. There is a need to explore other treatments that can be performed on cheese, especially while in their final retail packaging, to prevent further recontamination or growth of organisms already present. Currently, there is a growing interest in the use of different inexpensive nonthermal treatments for food preservation on finished products [3]. These treatments include the use of ionising radiation such as gamma [4], ultraviolet [3] and X-rays [5]. Food irradiation has been approved in many countries and has proven to be safe, with the potential for use in the preservation and extension of cheese product shelf-lives while maintaining their nutritional and sensory characteristics [4,6].

Producers should be informed about alternative methods of improving food safety considering their financial ability. However, there is limited information on the practicality and affordability of using irradiation treatment, especially for small-scale artisanal cheese producers. This study will focus on gamma irradiation treatment applied on retail cheese sample sizes. Gamma irradiation is a process in which food products are exposed to ionising radiation in the form of gamma-rays in controlled dosages to destroy pathogenic microorganisms [4]. The ionising radiation is used to generate highly active chemical reactive species, including free radicals such as the hydroxy radical, and hydrogen peroxide, which react with microbial DNA, thereby killing or injuring food-spoilage bacterial organisms [7].

Food irradiation treatment does not cause adverse organoleptic changes or serious side effects when applied appropriately [8]. Higher doses of radiation have adverse effects on the sensorial and nutritional qualities. Li et al. [9] noted a significant increase in benzyl methyl sulfide and lipid oxidation, with doses over 7 kGy. On the other hand, several cheese types have been treated successfully with doses below 5 kGy to enhance their shelf-life and product safety, and these includes Palmita types of cheese Queso blanco [10], Karish [11] and Ras cheese [12]. Adiel Pietranera et al. [13] also reported a dosage of 3 kGy to be effective in reducing *Staphylococcus* spp., coliforms, yeasts and moulds in ice cream. Recently, other authors [14,15] have also reported a significant reduction in microbial counts in foods after the gamma irradiation treatment.

For food safety purposes, it is important to focus on eliminating spoilage and pathogenic microorganisms such as *Enterobacteriaceae*, *Listeria monocytogenes*, *Escherichia coli* and coliforms on cheese products. Several types of artisanal cheeses are produced in South Africa and are characterised by a shorter ripening time of 90 days with a semihard consistency [2]. Most of these cheeses are from artisanal small-scale dairy plants. Appropriate treatment processes should be used in order to yield artisanal cheeses with improved health-promoting properties and consumer acceptance [16]. The specific objective of this study was then to evaluate the efficacy of gamma irradiation treatment applied to different cheese sample sizes of an artisanal pecorino-style cheese against *L. monocytogenes*, *E. coli*, coliforms and aerobic colony counts (ACCs). At the same time, the effects on cheese compositional, texture, colour and odour properties were evaluated. Furthermore, all costs involved for the treatment were also quantified.

2. Materials and Methods

2.1. Cheese-Making Process

A pecorino-style cheese was produced under typical artisanal processing conditions. Three different cheese batches were processed using the procedure previously reported by Branciari et al. [16], with minor modifications. Briefly, the cow milk was pasteurised by heating to 72 °C under continued stirring using the disc mixer to prevent the milk from burning at the bottom. After reaching 72 °C, the milk was then cooled to 35 °C with ice and cold water in a 200-L open dish container. *Lactobacillus delbruekii* spp. *lactis* and *Streptococcus thermophilus* bacteria culture (SACCO; MWT 0316, Cadorago, Italy) was added after cooling. A 50-g culture sachet with 10 UC (units) was mixed gently with 2 L of milk, and only 10% (200 mL) was added to the 80-L milk. Following culture addition after 25–35 min at 32 °C and pH of 6.5, the milk was coagulated by adding a natural animal rennet (94% chymosin and 6% bovine pepsin; Caglificio Clerici: A020782A, Cadorago, Italy) to 4 g/80 L of milk. The floating fat was removed before rennet addition. After 20 min of rennet addition, curd formation or density was checked with two fingers; when it felt denser between 25 min and 30 min, the curd was crossed with a curd cutter. Following this step, the curd was cut into small cubes of 0.5 cm^3. The cut curd in whey was continuously stirred with a whisk for 5–10 min to facilitate acid development and whey separation from the curds. The curds were then packed into moulds and manually pressed with clean and sanitised hands. Moulds were then covered with a thick plastic, and warm (35 °C) whey or water was constantly poured around the moulds to allow acid development and fermentation.

The curds were left to rest in moulds to allow whey drainage at room temperature (20 °C) for 24 h before salting. Cheese pH was constantly monitored before salting with regular turning. The cheese blocks were taken out from the moulds when the pH was 5.2, and crystallised fine-grained cheese salt (NaCl) was sprinkled on the surfaces. The cheese blocks (1.5 kg) were aged in a controlled chamber with temperature between 10 and 12 °C and relative humidity 80–90%. At day 60, the cheese blocks were aseptically cut into two different sample sizes of 250 and 500 g, and a microbiological analysis was conducted on eight samples from each processing batch ($n = 24$). After the analyses, all samples, either positive for *E. coli* ($n = 4$) and positive for *L. monocytogenes* ($n = 4$) or negative for *E. coli* ($n = 4$) and *L. monocytogenes* ($n = 4$), were selected. The samples were vacuum-sealed in polyethylene bags (Severin vacuum sealer bags 20 × 30 cm, Yuppiechef Online (Pty) Ltd., Cape Town, South Africa) with a low permeability for gases. The samples were then transported for irradiation treatment in a mobile refrigeration unit maintained at 4 °C.

2.2. Irradiation Treatment

The irradiation process was carried out using the Cobalt-60 gamma irradiator. The 250 g and 500 g samples were exposed to a maximum dosage of 5 kGy at a dose rate of 1 kGy/h and source strength of 150 kCi. A dose of 5 kGy maximum was predetermined based on the previous literature as an optimum dose that retarded the growth of microorganisms with a lower effect on the physicochemical and sensory properties [12,17,18]. The temperature was maintained at 4 °C during treatment according to the storage temperature. This prevented any change in temperature from affecting the planned microbial and physiochemical analyses. After the treatment, the samples were grated using autoclaved equipment, vacuum-packed and stored at −20 °C until a composition analysis was performed within a month of the treatment. Microbiological, texture, colour and volatile organic compound analyses were conducted immediately after the irradiation treatment.

2.3. Cheese Compositional Analysis

A cheese composition analysis ($n = 24$) was conducted on samples treated at 0.0 kGy (control) and 5.0 kGy. All frozen samples were thawed at room temperature before each respective analysis. The moisture and ash contents were determined according to Wehr and Frank [19]. Titratable acidity and pH were measured using the titratable acidity meter (Model HI 84529: Hanna Instruments, Cape Town, South Africa). The sodium chloride (salt) content was determined using the chloride analyser (Model 926 Sherwood Scientific, Cambridge, UK). The total fat percentage was evaluated according to Folch et al. [20]. The total nitrogen (N) content was quantified using the Dumas method with a macro-nitrogen analyser (LECO® FP528, LECO Corporation, Miami, FL, USA). The N content was multiplied by 6.38 to convert it to a protein compound [21]. The five major milk proteins (α-lactalbumin, β-lactoglobulin, α-casein, β-casein and k-casein) were analysed individually on the 2100 Bioanalyzer using the Protein 80 kit (Edition 07/2013: Agilent Technologies, Waldbronn, Germany). The individual proteins were expressed as a percentage of the total proteins in the sample.

2.4. Texture and Colour Analysis

Cheese texture was measured using a Texture Profile Analysis with an Instron Universal Testing machine equipped with Bluehill Texture Analyser software (3344 Model, Norwood, MA, USA) according to Neocleous et al. [22]. Briefly, 50 g of cheese blocks ($n = 24$) were left at room temperature for 1 h prior to analysis. The samples were cut into cubes of 2 cm × 2 cm × 2 cm, and five cheese cubes were tested for each sample. The samples were subjected to a double compression cycle, and up to 50% of the original sample height was compressed with an aluminium cylinder probe (diameter 3.6 cm). A crosshead speed of 1 mm/s was applied with a load cell of 5000 N. Cheese hardness (N) was recorded as the maximum force used during the first compression cycle. Springiness (%) was defined as the extent to which the sample returned to its original height between the first and second

compressions [23]. Cohesiveness was calculated as a ratio; that is, the energy (work done) of cheese during the second compression in relation to the first compression. Chewiness (J) was calculated as a product of hardness*cohesiveness*springiness [24].

Colour measurements were performed directly on the cheese blocks surfaces. Measurements were taken 3 times at different locations per sample. The lightness (L*), redness (a*) and yellowness (b*) parameters were recorded using the CIELAB colour meter: Colour guide 45°/0° colorimeter (BYK-Gardner GmbH, Gerestried, Germany) with an 11-mm diameter aperture using an illuminant/observer of D65/10° observer settings. The hue angle (H°) and chroma (C) levels were calculated as:

$$\text{H}° = \tan^{-1}\left(\frac{b*}{a*}\right) \times 57.29 \text{ (expressed in degrees); } \text{C} = \sqrt{\left(a*^2 + b*^2\right)}.$$

2.5. Smart Nose Analysis

The Smart Nose system was used to analyse samples (*n* = 24) of 500 g (*a*1) and 250 g (*a*2) at 5.0-kGy and at 0.0-kGy (*b*) gamma irradiation treatment. The Smart Nose technique was performed as explained in detail by Rapisarda et al. [25].

2.6. Extraction and Detection of Odour Active Compounds

Odour active compounds (OACs) were analysed by a Gas Chromatography/Olfactometry (GC/O) system to study the flavour profile of samples at 0.0 kGy and 5.0 kGy. Only two samples (named 278 and 369) out of a total of eight were randomly selected as representative of *b* and *a* clouds of the samples shown in the Smart Nose score plot. Odour active compounds were extracted twice by static headspace solid-phase microextraction (SPME) fibres with a 50/30-μm DVB/CAR/PDMS coating (Supelco, Bellefonte, PA, USA). Four grams of cheese sample were extracted following the method explained by Rapisarda et al. [26] and then analysed using an Agilent 6890N Series GCO system (Santa Clara, CA, USA). The identification of the volatile compounds was performed by an Agilent 5975C Mass Selective Detector (Santa Clara, CA, USA) (triple axis) at the same GC/O conditions. Volatile compounds were also identified and corroborated using the Flavornet internet database [27] to confirm the odour perceptions of each of the odour active compounds. Chromatographic and olfactometry methods were reported by Rapisarda et al. [25].

2.7. Microbiological Analysis

Microbiological analysis was done on the previously grated 500-g and 250-g samples according to AOAC [28]. Briefly, ACCs were determined by plating on ACC Petrifilms (3M, Johannesburg, South Africa). Coliforms and *E. coli* counts were enumerated by plating samples on *E. coli*/Coliform Petrifilms (3 M, Johannesburg, South Africa). All plates were incubated at 32 ± 1 °C for 48 h. *Listeria monocytogenes* were isolated using the rapid *L. monocytogenes* plates (Agar/Gelose) with the in vitro test (Bio-Rad, Marnes-la-Coquette, France). Bacterial counts were converted to logarithmic form for statistical analysis.

2.8. Statistical Analysis

The effect of γ-irradiation treatment (nonirradiated vs. irradiated) and different sample sizes (250 g and 500 g) on the cheese quality (compositional, colour, texture, milk proteins and microbial count) were analysed using the Glimmix procedure of Statistical Analysis System v.9.2 (2012; SAS Institute, Inc., Cary, NC, USA). Irradiation treatment and sample weight were treated as fixed factors and farm as a random factor. The Tukey–Kramer test was used to determine the differences among data means at the 5% significance level. A least squares mean comparison was done using groupings. Analysis of variance was done using the following model:

$$Y_{ijk} = \mu + D_i + V_j + (DV)_{ij} + \varepsilon_{ijk},$$

where Y_{ijk} is the cheese quality, μ is overall mean, D is effect of the ith irradiation treatment, V_j is the effect of the jth sample size, (DV) is interaction of the ith irradiation treatment and jth sample size and ε_{ijk} is the residual error.

Principal component analysis (PCA) was performed for all datasets obtained from Smart Nose software 1.51. The PCA gives the possibility of making a group assignment by Euclidean distances in the multidimensional space created by the PCA. For each separation pattern, a new set of parameters was chosen to calculate the PC scores. The volatile organic compound presence or absence ratio between the irradiated and nonirradiated samples was analysed with Fisher's exact test.

3. Results and Discussion

3.1. Compositional Properties

Similar to other food processing techniques, irradiation can induce certain alterations that can modify the chemical composition and nutritive values of food [10–12]. In the current study, the moisture content, water activity, total N (TN), protein, pH and fat in DM, salt and salt in moisture were reduced ($p < 0.05$) by the irradiation treatment (Table 1). The noted differences were not significant for other studies [10,12], and this could be due to the differences in the irradiation dose applied and chemical composition of different cheese varieties. The reduction in the moisture content could be due to the decrease in water-holding capacity of casein proteins as a result of irradiation [10–12]. This has a possible effect on the water activity, pH, TN and fat in the DM content. The noted reduction in TN % could be due to the destructive effect of irradiation on the natural flora and milk enzymes subsequently affecting the protein content [29]. The fat content was not affected, similar to what is shown in other studies where macronutrients such as lipids were reported not to be significantly affected by ionising radiation doses of up to 10 kGy [30,31].

Table 1. Composition (LSM [a] ± SEM [b]) of artisanal pecorino-style hard cheese ($n = 24$) at 0.0-kGy and 5.0-kGy dosages of the irradiation treatment.

Parameter	Dosage		p-Value
	0.0 kGy	5.0 kGy	
Moisture%	30.3 ± 1.14	28.6 ± 1.16	0.0091
Ash %	3.75 ± 0.46	3.78 ± 0.46	0.7350
pH	5.16 ± 0.06	5.11 ± 0.06	0.0287
Lactic acid%	0.86 ± 0.11	0.87 ± 0.12	0.8485
Protein% of TN [c]	30.4 ± 1.77	29.6 ± 1.78	0.0084
TN %	4.76 ± 0.28	4.63 ± 0.28	0.0082
Fat%	32.0 ± 1.20	30.7 ± 1.23	0.0580
Fat in dry matter%	45.9 ± 2.09	43.0 ± 2.11	0.0007
Water activity	0.93 ± 0.01	0.92 ± 0.001	0.0001
Salt%	2.69 ± 0.52	1.95 ± 0.53	0.0001
Salt in moisture%	9.19 ± 2.27	7.18 ± 2.28	0.0001
Milk proteins			
α-lactalbumin%	11.5 ± 1.78	11.2 ± 1.80	0.6219
β-lactoglobulin%	14.2 ± 1.4 2	12.4 ± 1.36	0.1212
α-casein%	25.2 ± 3.79	22.5 ± 3.86	0.1824
β-casein%	3.81 ± 0.71	0.83 ± 0.63	0.0024
k-casein%	11.3 ± 1.37	9.48 ± 1.44	0.1432

[a] LSM = Least square means; [b] SEM = standard error of the means; [c] TN = Total Nitrogen; $p < 0.05$. * Values correspond to the average of 250 and 500-g samples.

All milk proteins, except for the β-casein, were not affected ($p > 0.05$) by the irradiation treatment (Table 1). The amount of β-casein decreased ($p < 0.05$) at the 5.0-kGy dosage. Ham et al. [32] reported a decrease in α and β-caseins in both milk and Queso Blanco cheese after treatment. Seisa et al. [17] postulated that differences in the development of the peptide profile of irradiated cheddar cheese are due, in part, to protein degradation

by radiation. It is important to elucidate the effect of irradiation on the changes of specific milk proteins, since the irradiation treatment can be used for the elimination of allergenic proteins in foods [32,33]. These studies have, in fact, demonstrated that food irradiation can reduce food allergies by eliminating specific allergenic proteins. The composition properties were not different ($p > 0.05$) between the 250 and 500-g samples at a dosage of 5.0 kGy (data not shown).

3.2. Colour and Texture Properties

All colour parameters decreased ($p < 0.05$) at 5.0-kGy treatment dosage, except the chroma values (Table 2). Lower lightness (L*) values can be present because of the browning reactions that may occur during irradiation due to lipid oxidation or Maillard reaction [18]. Similar to this study, Seisa et al. [17] also reported a decrease in yellowness for cheddar cheese after gamma irradiation. The redness (*a**) values decreased ($p < 0.05$) in agreement with what was observed by Kim et al. [34] for sliced and pizza cheeses. Conversely, Konteles et al. [18] have reported an increase in the *a** values for Feta cheese. These differences across studies can probably be related to fat and moisture content of the different cheese varieties. The decrease in yellowness and red appearance can be due to the degradation of riboflavin and β-carotene by irradiation in the cheese structure [18].

Table 2. Effects of the irradiation treatment (0.0 kGy and 5.0 kGy) and sample size (500 g and 250 g) on colour properties (LSM [a] ± SEM [b]) of artisanal pecorino-style hard cheese (*n* = 24).

| Property | Treatment | | | | *p*-Values | | |
| | Dosage (D) | | Weight (W) | | | | |
	0.0 kGy	5.0 kGy	250 g	500 g	D	W	D × W
[c] L*	75.7 ± 2.50	73.1 ± 2.49	74.2 ± 2.49	74.6 ± 2.49	0.0005	0.6042	0.6042
[d] *a**	4.36 ± 0.72	1.42 ± 0.72	2.84 ± 0.72	2.94 ± 0.72	0.0001	0.5587	0.5587
[e] *b**	23.8 ± 3.03	21.7 ± 3.03	22.8 ± 3.03	22.8 ± 3.03	0.0001	0.9860	0.9860
Chroma	79.9 ± 1.24	87.0 ± 1.26	83.7 ± 1.25	83.2 ± 1.24	0.0001	0.3487	0.3487
Hue angle (°)	24.3 ± 3.06	21.8 ± 3.06	23.0 ± 3.06	23.0 ± 3.06	0.0001	0.9997	0.9997

[a] LSM = Least square means; [b] SEM = standard error of the means; [c] L* lightness, [d] *a** redness and [e] *b** yellowness; $p < 0.05$.

Chroma (C) values that quantify the saturation or colour intensity increased ($p < 0.05$) at the 5.0-kGy irradiation treatment dosage, while the hue angle (H°) decreased. The increase in C indicates that the purity of colours was greater at 5.0 kGy. The H° values for both irradiated and nonirradiated cheese samples were closer to 0°, which represents a more intense red colour. Similar to this study, Kortei and Akonor [35] also reported a linear correlation between the L* and H° values in irradiated mushroom samples. No differences ($p > 0.05$) were noted for all the colour parameters between the 250 and 500-g samples at a 5.0-kGy dosage (Table 2).

Cheese cohesiveness and springiness decreased ($p < 0.05$) at the 5.0-kGy irradiation treatment (Table 3). These results could be due to the effect of the treatment on the cheese surface, thus the aggregation of the proteins causing changes in the rheological behaviour [36]. The reduction in the moisture content at 5.0 kGy could have affected both the hardness and chewiness values (Table 3). The cheese hardness increases as the moisture content decreases, consequently affecting the springiness and chewiness values [24,37]. Additionally, the denaturation of milk proteins and enzymes may occur with irradiation, thereby also bringing about textural changes [38]. The 250-g samples had higher ($p < 0.05$) hardness values at 5.0 kGy compared to the 500 g (Table 3). On the other hand, the springiness values were noted to be higher in the 250-g samples corresponding to changes in the hardness. This suggests that the treatment had more effect on the texture properties of smaller samples (250 g) than 500 g. In contrast, Konteles et al. [18] reported no irradiation effects on Feta cheese texture; this could be due to the differences in the chemical compositions and varieties of cheeses examined.

Table 3. Effects of the irradiation treatment (0.0 kGy and 5.0 kGy) and sample size (500 g and 250 g) on the texture properties (LSM [a] ± SEM [b]) of artisanal pecorino-style hard cheese (*n* = 24).

| Property | Treatment | | | | *p*-Values | | |
| | Dosage (D) | | Weight (W) | | | | |
	0.0 kGy	5.0 kGy	250 g	500 g	D	W	D × W
Hardness (N)	182.9 ± 70.31	209.0 ± 70.44	234.4 ± 71.04	183.6 ± 70.92	0.0294	0.0049	0.0332
Cohesiveness	1.26 ± 0.11	0.44 ± 0.11	0.44 ± 0.14	0.43 ± 0.13	0.0001	0.9852	0.9889
Chewiness (J)	1132.9 ± 316.24	448.2 ± 319.67	495.1 ± 334.76	401.2 ± 331.96	0.0001	0.7114	0.7114
Springiness (%)	53.8 ± 1.87	42.3 ± 1.92	45.0 ± 2.11	39.6 ± 2.07	0.0001	0.0018	0.0018

[a] LSM = Least square means; [b] SEM = standard error of the means; *p* < 0.05.

3.3. Smart Nose

The Smart Nose analysis was used to detect differences in the volatile profile of the samples at the 0.0-kGy (*b*) and 5.0-kGy (*a*1 and *a*2) dosages. The score plot (Figure 1) showed a wider separation (PC1: 68.79%; PC2: 25.27%) both between the *b* and *a* samples, as well as between the *a*1 and *a*2 groups. Furthermore, aroma profiles were determined on two samples (identified as samples 278 and 369), including the respective subsamples, based on the results from the Smart Nose analysis (Table 4). The Gas Chromatography Olfactometry results showed a higher frequency of ketone compound presence than the other chemical classes in both samples 278 and 369 (Table 4). The Ketone class is usually formed in heat-processed milk, and they are the major contributors to their cooked odour perception [39,40]. Diacetyl could be formed by α-dicarbonyl, an intermediate compound formed during nonenzymatic browning reactions. Terpenes are derived from secondary plant metabolites, and they were not affected by the treatment. A study on the plants present in the mountain area demonstrated that the pastures are rich in terpenes, especially monoterpenes [41]. The terpenes have an important chemical property represented by chirality, and therefore, different enantiomers of the same molecule may differ in taste, aroma or bioactivity.
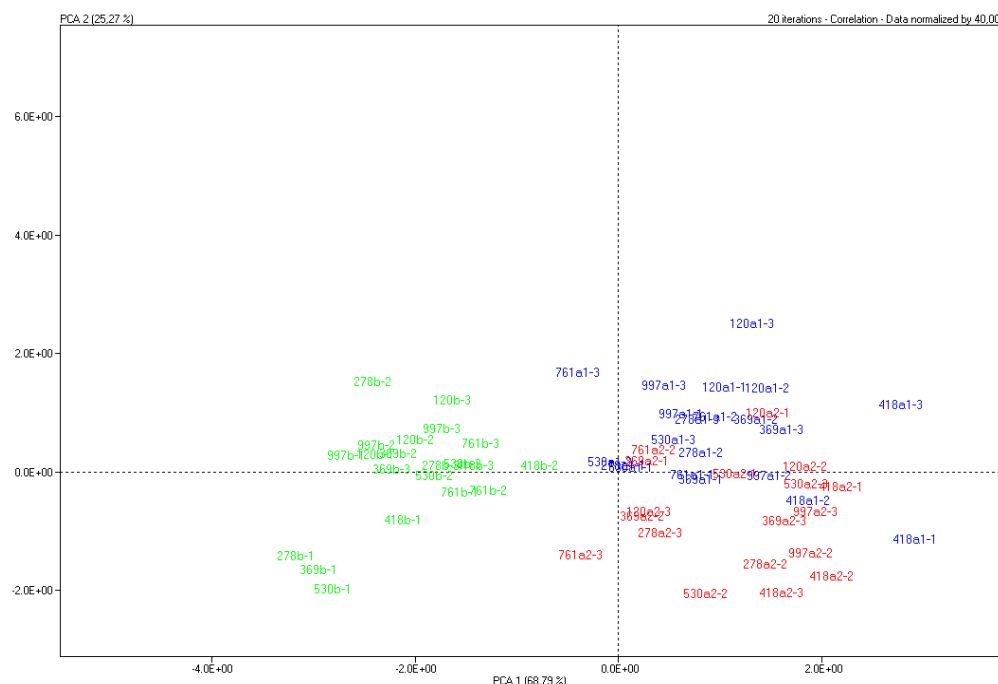

Figure 1. Score plot of artisanal pecorino-style hard cheese of pieces of 250 and 500 g (*n* = 24) at 0.0−kGy and 5.0−kGy irradiation treatment. Green = Samples "0.0 kGy"; Red (a2) = 5.0 kGy, 250 g; Blue (a1) = 5.0 kGy, 500 g.

Table 4. Odour Active Compounds of artisanal pecorino-style hard cheese samples at the 0.0-kGy and 5.0-kGy radiation dosages.

Compounds	Chemical Class	Odour Perception	LRI [a]	Ident [b]	278			369		
					b [c]	*a*1 [d]	*a*2 [e]	*b*	*a*1	*a*2
Methylbutyric acid	Acid	Cheese, Butyric	367	PI	X					X
Total acid					*1*	*0*	*0*	*0*	*0*	*1*
Nonanal	Aldehyde	Green	1103	PI				X		
2-Nonenal	Aldehyde	Green	1143	PI	X					
2,6-Nonadienal	Aldehyde	Hay	1149	PI	X					
Total aldehyde					*2*	*0*	*0*	*1*	*0*	*0*
Safrole	Aromatic Hyd	Unpleasant	1280	PI		X	X			
Total aromatic hydrocarbon					*0*	*1*	*1*	*0*	*0*	*0*
Ethyl butyrate	Ester	Apple	798	PI	X	X		X	X	X
Ethyl methylbutyrate	Ester	Orange	842	PI				X	X	
Ethyl octanoate	Ester	Wine, Fruity	1185	PI	X	X	X	X	X	X
Total ester					*2*	*2*	*1*	*3*	*3*	*2*
Diacetyl	Ketone	Butter	639	PI	X	X	X	X	X	X
Heptanone	Ketone	Sweet	895	PI		X	X			
1-Octen-3-one	Ketone	Mushroom	973	PI	X	X	X		X	X
2-Nonanone	Ketone	Hot milk	1082	PI	X	X	X		X	X
3,5-Octadien-2-one	Ketone	Mushroom	1095	PI		X	X		X	X
Total ketone					*3*	*5*	*5*	*1*	*4*	*4*
Acetylpyrazine	Pyrazine	Roast, Rancid	1027	PI					X	X
Total pyrazine					*0*	*0*	*0*	*0*	*1*	*1*
Thiophene	Sulfur	Garlic	665	PI		X	X			
Methional	Sulfur	Potato	905	PI	X				X	X
Butyl isothiocyanate	Sulfur	Garlic	956	PI	X	X	X	X	X	X
Total sulfur					*2*	*2*	*2*	*1*	*2*	*2*
2,3-Dehydro-1,8-cineole	Terpene	Orange	990	PI	X	X	X	X	X	X
(Z)-Linalool oxide	Terpene	Wine, Fruity	1073	PI	X	X	X	X	X	X
(+)-cis-Rose oxide	Terpene	Wine, Fruity	1112	PI	X	X	X	X	X	X
Total terpene					*3*	*3*	*3*	*3*	*3*	*3*
Total compounds					**13**	**13**	**12**	**9**	**13**	**13**

[a] Linear Retention Index with a hHP-5 column. [b] Identification: PI, comparison with LRI flavour database. [c] The 0.0-kGy irradiation treatment. [d] The 5.0-kGy irradiation treatment at 500 g. [e] The 5.0-kGy irradiation treatment at 250 g.

Observing 278 and 369 samples at the 0.0-kGy gamma irradiation dosage, 278 samples showed the highest number in AOCs. The differences in AOCs were not only in the number but also in the qualitative volatile compounds. Considering the groups (*b* and *a*) and subgroups (*a*1 and *a*2) of the same sample, 278 samples did not show differences in number of AOCs among the *b*, *a*1 and *a*2 samples, whereas 369 samples showed a fewer number of AOCs in the *b* and *a* samples. In detail, 278 samples had methylbutyric acid, 2-nonenal, 2,6-nonadienal and methional-like unique compounds in the *b* vs. *a* groups. On the other hand, safrole, heptanone, 3,5-octadien-2-one and thiophene were absent in the *b* group but present in both the *a*1 and *a*2 subgroups. Safrole and thiophene are responsible for the unpleasant garlic odour perception. Another possible problem with the irradiation treatment is that animal [42,43] in vitro and in vivo studies [44,45] have confirmed that

safrole is carcinogenic. Huo et al. [46] also reported unfavourable alterations in the cheese odour when samples are irradiated at doses of 1.51 kGy and 2 kGy. Free radicals are produced during the irradiation treatment, which might trigger lipid and/or protein oxidation, leading to the production of secondary oxidation products such as alkanes, alkenes, aldehydes, alcohols, ketones and acids [47]. Secondary oxidation products are also broken down into volatile compounds and/or undergo polymerisation, cyclisation and isomerisation that might give off a fishy, metallic, rancid and oxidised odour perception [48].

By comparing the presence/absence of specific compounds in the two samples, 369 had methylbutyric acid only in subgroup *a*2, different from sample 278 that, as said before, were present in the *b* group. Moreover, 1-octen-3-one and 2-nonanone were present in the *a*1 and *a*2 subgroups, different from sample 278, for which they were present in all groups. Methional was also present in the *a*1 and *a*2 subgroups and absent in *b* in the opposite condition compared to sample 278. Another compound, 3,5-octadien-2-one, was present in both *a*1 and *a*2 subgroups and absent in *b* in both samples 369 and 278. The differences in the number and type of volatile compounds between samples 278 and 369 could explain the high variability and, consequently, the separation of the *b*, *a*1 and *a*2 samples in the Smart Nose score plot. From the observations of the data, it could be hypothesised that the γ-irradiation treatment influenced in different ways the volatile profile of the two samples. Differences in the volatile compounds have an influence on the cheese sensory properties, as demonstrated by Seisa et al. [17]. Further quantitative investigations are needed to understand the weight each compound had in determining the variability between the *b* and *a* groups, including subgroups *a*1 and *a*2, as demonstrated by the Smart Nose analysis.

3.4. Microbiological Quality

The numbers of all the tested microorganisms were reduced ($p < 0.05$) by the irradiation treatment (Figure 2) to below the recommended European Union limits (<2.0 $Log_{10}CFU/g$ *E. coli*, <4.0 $Log_{10}CFU/g$ coliform and <7.0 $Log_{10}CFU/g$ ACCs) for "ready-to-eat" cheese products [49]. Additionally, Seisa et al. [17] and Konteles et al. [18] reported a significant decrease in the total bacteria counts after the irradiation treatment. Aly et al. [11] further noted that the microbial counts of the irradiated samples decreased with an increase in the irradiation dose from 1 to 5 kGy, with a complete reduction of the coliform counts at 5 kGy. The inactivation of food pathogens by irradiation is due to cellular membrane or DNA damage, causing sublethal injury preventing multiplication and can also cause death [50].

Listeria monocytogenes are also of importance, as they are frequently reported as post-process contaminants in ready-to-eat food products, including meats, sandwiches and cheeses [2,51]. They can grow at refrigeration temperatures and during aging. For the current study, at 5.0 kGy, all *L. monocytogene*-positive (16%) samples tested negative. Corresponding with our results, Kim et al. [34] also observed a total reduction in the number of *L. monocytogenes* at 5 kGy, and Konteles et al. [18] reported permanent damage inhibiting further growth even during storage.

The stage at which the contamination would have occurred is also important. Konteles et al. [18] noted that, when milk is contaminated after pasteurisation, even a higher irradiation dose of 4.7 kGy was not enough to eliminate the pathogenic bacterium compared to contamination at the surface of cheese during packaging. At 5.0 kGy, the 500-g samples had significantly higher (1.51 $Log_{10}CFU/g$) ACC counts compared to the 250-g (0.79 $Log_{10}CFU/g$; Figure 3). However, the coliforms and *E. coli* counts were not different ($p > 0.05$) between the 250 and 500-g samples for the 5.0-kGy treatment. These results proved that the irradiation treatment can significantly reduce the microbial counts on retail cheese samples with a size below 500 g without adverse effects of the physicochemical properties. Further studies should focus on using bigger retail sizes and note if the treatment will have the same level of efficacy. An additional sensory analysis should also be done to determine if there are any effects on consumer acceptability.
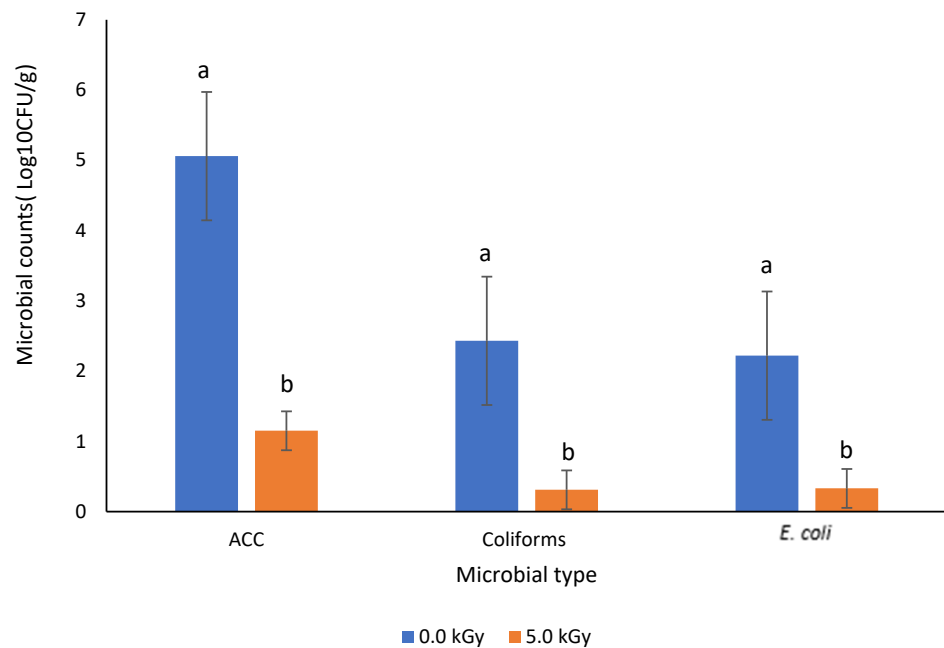
Figure 2. Microbial counts at the 0.0-kGy and 5.0-kGy irradiation treatments of artisanal pecorino-style hard cheese (*n* = 24). Different superscripts indicate a difference (*p* < 0.05). ACC: Aerobic colony counts; *E. coli*: *Escherichia coli*.
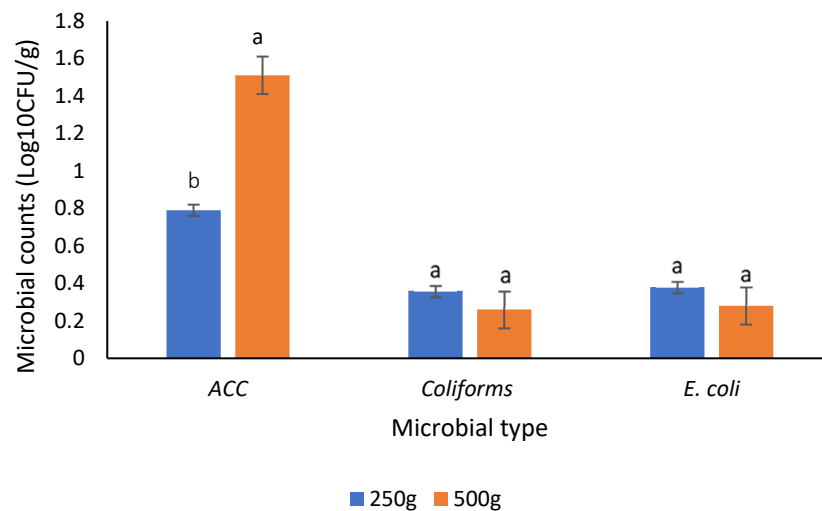

Figure 3. Microbial counts of artisanal pecorino-style cheese of pieces of 250 and 500 g at the 5.0-kGy irradiation treatment (*n* = 24). ACC: Aerobic colony counts; *E. coli*: *Escherichia coli*.

3.5. Costs Evaluation

For resource-limited small-scale producers, it is important to make use of low-cost accessible technologies for cheese processing and microbiological treatment [52]. It is expected that, in the near future, nonthermal technologies will be the best affordable alternative to improve food safety and allergenic properties [33]. The irradiation treatment cost is calculated based on the density of the sample to be treated and maximum dosage to be applied. The consignment for this study cost was US $31.20 for 14.5 kg (sum of 250 g and 500 g cheese pieces). The cheeses were vacuum-sealed in polyethylene bags, and the packaging costs US $0.59 for one bag (20 × 30 cm). Polyethylene bags with low permeability to gases are normally used, and samples should not be exposed to oxygen during the treatment to reduce the chances of oxidation that might result in off flavours. It is important to note that smaller packages of <500 kg tend to be relatively expensive;

the cost decreases when a bigger consignment is treated (1000 kg). As of August 2018, in South Africa, the minimum cost for each consignment stack of 1 m × 1.2 m × 1.5 m in height and maximum weight of 1000 kg was US $27.13 (US $0.027/kg), excluding vat. Small-scale producers can work as cooperates so as to have the maximum weight of 1000 kg and benefits from the irradiation low costs. According to the above figures working with a maximum weight of 1000 kg, the cost of packaging bags and the irradiation treatment gives an estimate of US $0.62 per kilogram of cheese. The evaluated costs for the treatment are relatively affordable for small-scale producers in case they produce cheese suspected or known to be contaminated [52]. The cheese products should be treated in their final packaging to prevent further contamination and limit packaging costs.

4. Conclusions

The irradiation treatment was able to effectively reduce *E. coli*, coliforms, ACC and *Listeria* spp. counts below the recommended limit for food safety on the cheese samples. Although the results suggest a probable influence of irradiation treatment on the odour active profile of cheese, the treatment did not cause adverse effects on cheese physicochemical properties, and it was noted to be affordable. All this justifies the feasibility of the gamma irradiation treatment for small-scale producers. However, producers still need to process the cheeses following appropriate hygiene protocols and also avoid posttreatment contamination. More studies need to be done on consumer perceptions and acceptability regarding irradiated cheese, and efforts should be made to standardise the treatment process parameters.

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