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Artisanal fresh cheese quality improvement using hurdle technologies combination

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Abstract
The aim of this study was to compare the effect of combining electron beam irradiation with the activation of the lactoperoxidase system as a decontamination tool. Microbiological, physico-chemical and organoleptic analyses were carried out during storage of the cheeses at +4±1ºC for 18 days, and the shelf life was determined.
The use of electron beam irradiation combined with lactoperoxidase had a significant effect on microbiological quality compared with control cheeses and those activated by lactoperoxidase alone throughout the shelf life.
However, the activation of lactoperoxidase alone had no significant impact on the development of these germs. With regard to sensory analysis, no descriptor had a significant effect detected by the panel throughout the shelf life. On the other hand, these conservation combination enabled the the shelf life to be extended.
The control cheese had a use-by date of 3.63 days and the cheese preserved with lactoperoxidase only 3.09 days. The combination, low-dose electron beam irradiation with LPS extended the shelf life of cheese to 6.82 days, the medium dose (1kGy) combined with lactoperoxidase had the shelf life of 10.92 days compared with 16.93 days for the highest dose (1.5kGy) combined with lactoperoxidase.

Keywords: Microbiological quality, shelf life, electron accelerator, lactoperoxidase, organoleptic quality

Introduction

The cheese market is booming due to the constant increase in its production and consumption (Emma *et al*., 2016). Cheese is highly valued by consumers for its nutritional and organoleptic qualities as it is recognised as an important supplier of nutrients, and is rich in many minerals, including Ca, Mg, P and Zn, as well as vitamins A, D, E and K (Emma *et al*., 2016).
Dairy products are the main source of dietary calcium in many countries, including the US, UK and most of Northern Europe, providing a source that cannot be easily replaced by other foods (Emma *et al*., 2016).
On the other hand, cheese has a fairly short shelf life due to its multiple varieties of micro-organisms such as yeasts, *Pseudomonas sp*, heterofermentative lactic bacteria, *Clostridia*, *Bacillus sp*, coliforms, *Klebsiella pneumoniae*, *Penicillium* and psychrophilic spoilage microflora (Zhu *et al*., 2020) [22].
The application of heat treatments at very high temperatures can alter the composition of milk as it affects protein structures and water-soluble vitamins, resulting in a decrease in total fat and total solids and an increase in urea (Bezie, 2019) [3].
Alternative processes are therefore needed to solve this problem, mainly the use of combination technology (Peng *et al*., 2015) [15].
At the crossroads of major economic, environmental and public health issues, food preservation techniques today deserve the mobilisation of all stakeholders to ensure that technologies evolve towards solutions that are more respectful of both the environment and the consumer (Gontard *et al*., 2017) [7].
This makes the application of barrier technology very interesting as it can extend shelf life while preserving nutritional and sensory value. This technology works at lower temperatures and with shorter processing times, while ensuring microbiological safety (Predrag *et al*., 2020). The combination technique involves preserving food using several agents/methods applied simultaneously or sequentially (Peleg, 2020) [14].
The combination of different technologies and/or the adjustment of a food's pH, water activity (aW), oxygen tension, etc., can be used to improve its preservation efficiency, which has been widely documented and has become common knowledge (Peleg, 2020) [14]. The latter is widely used in industrialised as well as developing countries for efficient food preservation (Peng *et al*.,2015) [15].
Combination technology is an important approach that can be used to improve quality parameters during food processing and storage. Intelligent application of barriers improves sensory characteristics, food chemical and microbiological qualities (Peng *et al*., 2015) [15]. Moreover, more than 60 reported barriers are available, these can be used in different combinations and concentrations for a wide range of foods. This versatility makes the application of the technology possible in modern and local food processing (Peng *et al*., 2015) [15].
A particular example is the use of lactoperoxidase, a natural enzyme considered to be an important element in the host's natural defence system against bacterial infection. Indeed, the LPO system can also be used to increase the stability of milk storage at high ambient temperatures (Zarei *et al*., 2016) [20].
Non-thermal processes are implemented to inactivate spoilage micro-organisms and improve the nutritional, sensory and microbial characteristics of the product; a good opportunity that presents itself is the use of irradiation during cheese processing (Huo *et al*., 2013) [8].
Irradiation is the use of ionising radiation from radioactive isotopes of cobalt or caesium or from accelerators producing controlled amounts of beta rays or X-rays on food (Huo *et al*., 2013) [8].

Food irradiation has been identified as a safe technology on the food process and used to disinfect and preserve food, including extending shelf life (Huo *et al*., 2013) [8].

Materials and methods

Cheese manufacture

The cheese was produced from raw cow's milk. Subsequently, the standardized milk was pasteurized at 85°C/10 min, cooled to 32 C, and inoculated by adding 0.125 g/l of mesophilic starter culture (Barukcic *et al*., 2020) [2]. Fermentation (acid coagulation) of the milk was carried out at 30 C for about 16 h until curd was formed and pH of about 4.6 was reached (Barukcic *et al*., 2020) [2].

At this point, the fermentation process was interrupted by rapid cooling. The resulting curd was cut by a sterile knife and heated to a maximum of 40°C while continuing agitation to allow separation of the whey. Transferred to molds in cold storage (15-16°C) and drained (Barukcic *et al*., 2020) [2].

The cheese yield is calculated according to the following formula:

$$Y\% = \frac{Wc}{w} * 100$$

Y : means the cheese yield in %.
Wc : means the weight of cheese obtained in kilograms.
W : means the weight of the milk in kilograms.

LPS activation

The sample was subjected to refrigeration combined with activation of the LP system by addition of sodium thiocyanate (NaSCN) as a source of thiocyanate (SCN) to a final concentration of 14 mg⁄L (Boulares *et al*., 2011) [4].

After 1 min of thorough mixing of the milk, 30 mg ⁄L of sodium percarbonate ($2Na_2CO_33H_2O$) was added as a source of hydrogen peroxide (H_2O_2) as recommended by the International Dairy Federation (IDF1988).

Technique of combining LPS with irradiation

This technique consists in activating the milk with LPS which will be used for the manufacture of cheese, which will be subsequently irradiated. The cheeses were irradiated in the National Center for Nuclear Science and Technology (CNSTN) of Sidi Thabet of Tunisia using a gas pedal 10 MeV electron beam at doses of 0.5; 1; 1.5 kGy respectively (Huo *et al*., 2013) [8].

The samples were distributed as follows

Samples	Control	LPS	LPS+ 0,5 kGy	LPS+ 1 kGy	LPS+ 1,5 kGy

All the tests are spread over three periods: t_0, t_9 and t_{18}.

Physicochemical analysis

pH

The pH is measured with a pH meter (KDD002) to 0.01 units of accuracy. For milk the determination is made on 10 mL of the sample. For cheese, the electrode of the pH meter is placed directly in the cheese (Fedala *et al*., 2020) [6]

Titratable acidity

This is an acid-base titration, lactic acid is neutralized by a solution of sodium hydroxide NaOH in the presence of phenolphthalein as a colored indicator ((Fedala *et al*., 2020) [6].

For milk the acidity is expressed in degree Dornic (°D). The titration is performed by an alkaline solution (NaOH, N/9) in the presence of phenolphthalein at 1% (w/v), (1 mL of NaOH (N/9) corresponds to 0.01 g of lactic acid per cent) (Fedala *et al*., 2020) [6].

Determination of fat

The method is based on the acid-butyrometric method of Van Gulik. It is based on the dissociation of cheese proteins by the addition of sulfuric acid and separation of the fat by centrifugation in a Van Gulik butyrometer.

In a Van – Gulik butyrometer, put 3g of cheese, add sulfuric acid so that it covers the cheese mass, making the proteins dissociate in a water bath. After complete dissociation, fill the graduated rod with sulfuric acid and add 1 ml of isoamyl alcohol (Fedala *et al*., 2020) [6]. The separation of fat is done by centrifugation (Fedala *et al*., 2020) [6].

The fat expressed in g/100g of cheese is obtained by direct reading on the butyrometer scale (Fedala *et al*., 2020) [6]

Determination of total dry extract

The total dry extract (TDE) is determined using an oven set at 103±2°C. The test samples are measured to the nearest 1 mg and the dry matter is expressed as a percentage by weight by the remainder after drying (Fedala *et al*., 2020) [6].

Two grams of cheese are weighed after homogenization of the cheese paste, and the dry matter is expressed as weight percentage by the remainder after drying (Fedala *et al*., 2020) [6].

$$\mathbf{TDE = E2 - MC \,/\, E1 \times 100}$$

TDE: total dry extract
E1: weight in grams of the capsule and the test sample.
E2: weight in grams of the capsule and residue after drying and cooling.
Mc: weight in grams of the empty capsule.

Determination of the dry defatted extract

It is the result of the difference between the total dry extract and the fat content and
determined as follows:

$$\mathbf{ESD = TDE - MG}$$

TDE : total dry extract
ESD: dry extract defatted
MG: Fatty matter

Measurement of moisture

The moisture is calculated by the following formula:

$$\mathbf{Moisture\ (\%) = 100 - TDE}$$

Rheological study

Hardness (N), of cheese was determined by texturometer analyzer type TVT 6700. Cheese samples were cut into 5 * 5 cm cubes and stored at a room temperature before measurements (Barukcic *et al*., 2020) [2].

Then the prepared samples were subjected to double compression at a traverse speed of 1 mm/s and a penetration distance of 40 mm up and down with 10 s between the two cycles (Barukcic *et al*.,2020) [2].

Sensory analysis

Once the production is finished, a hedonic test is carried out for the two cheeses. The purpose of this test is to compare the overall hedonic appreciation of the different cheeses by

focusing on the individual feelings related to the pleasure or displeasure caused by the food (Fedala *et al*., 2020) [6].
Consumer acceptance was determined using a 9-point scale. The results of the sensory analysis are represented by a radar graph. It allows to highlight the data around a reference value (Fedala *et al*., 2020) [6].

Microbiological analysis
The cheese was aseptically removed from the package and the surface was cut with a sterile knife. The cheese slices were weighed into a sterile beaker with 100 ml of sterile distilled water. The cheese was grated and homogenized thoroughly (Fedala *et al.*, 2020) [6]
Serial dilutions of the homogenates were placed on appropriate media in Petri dishes and analyzed immediately (Fedala *et al*., 2020). [6] The numbers of total coliforms, total aerobic mesophilic flora, yeasts and molds were monitored on consecutive days during storage (Fedala *et al*,. 2020) [6]
The media and incubation conditions used were as follows:
Coliforms: spread on violet red bile agar (VRBA), with a double cover layer of the same medium, incubated at 30° C for 24 to 48 hours (Fedala *et al*,. 2020) [6]
Total aerobic mesophilic flora: spread on PCA (Plate Count Agar) and incubation is done at 30°C for 48 hours (Fedala *et al*,. 2020) [6]
Yeasts and molds: spread on Sabouraud dextrose agar, incubated at 25° C for 5 to 7 days (Fedala *et al*,. 2020) [6]

The determination of shelf life
The general equation that describes the loss of quality of a food is applicable for any factor A is as follows:

$$r = \frac{d[A]}{dt} = K\,[A]n$$

r : rate of the degradation reaction = rate of formation of A ;
K : reaction rate constant or apparent rate ;
A : concentration of the factor to be followed;
n : order of the degradation reaction.

Statistical analysis
All experiments were repeated at least three times. The results were subjected to a one-factor analysis of variance with a significance level of 95% using Excel for the radar presented for the sensory analysis and by SPSS software using the one-factor ANOVA test.

Results and disscusion
Cheese yield
Cheese yield is of great interest in the cheese industry because it reflects the overall quantitative distribution of milk constituents during draining (Vignola *et al*., 2002) [19].
It requires a high level of dry extract and more specifically protein (casein) and high fat concentrations (Vignola *et al*., 2002) [19].
According to our results, we obtained a Cheese yield Y= 10.7%.

Physicochemical analyses
Effect of Hurdle technology on pH
No significant difference (P > 0.05) in pH was observed between the control and the LPS-activated cheese. Seifu *et al*, (2004) [17] stated that activation of cheese by LPS had no effect on pH. Similarly, no significant difference in pH was observed between the controls and the cheeses activated by LPS throughout the ripening period (Boulares *et al*., 2011) [4].
Fig 1 Shows the effect of ebeam treatment (0.5, 1 and 1.5 kGy) and LPS activation on cheese pH at (0 d) t_0, (9 d) t_9 and (18 d) t_{18} of storage at 4±1°C.
In fact, we noted that the pH of the control cheeses at t_9 and t_{18} and those activated by LPS decreased significantly compared to the other samples that underwent the combined technique.
This significant decrease can be attributed to the inhibition of the microbial flora and particularly the lactic acid bacteria responsible for the acidification of the cheese.
For the products treated with ebeam combined with LPS and for the low dose of 0.5 kGy, the pH fell from 4.51 ± 0.01 at t_0 to 4.47 ± 0.01 at t18, while for the medium dose of 1 kGy the pH fell from 4.49 ± 0.01 at t_0 to 4.45 ± 0.01 at t18.
Moreover, the highest dose, 1.5 kGy, reduced the pH of the cheeses studied from 4.48 ± 0.01 at t0 to 4.43 ± 0.01 at t_{18}.
This decrease in pH can be explained by the inhibition of the microbial flora and in particular the lactic bacteria responsible for the acidification of the cheese spread (Agherghour *et al*., 2015) [1].
The pH is a quality index that determines the ability of food to be preserved. It is one of the main obstacles that microbial flora must overcome in order to proliferate (Agherghour *et al*., 2015) [1].
It is therefore important to measure the pH in order to determine the stability of the food with respect to microorganisms, which are pathogenic to humans rarely develop at an acid pH, below 4 (Agherghour *et al*., 2015) [1].
Most microorganisms grow at pH close to neutral, whereas moulds grow at acid pH (Agherghour *et al*., 2015) [1].
Our results disagree with those of Kim (2010) [9] who studied the effect of ebeam with different doses of 1, 3 and 5 kGy on the pH of cheese and reported that electron beam irradiation also had no significant effect on pH.

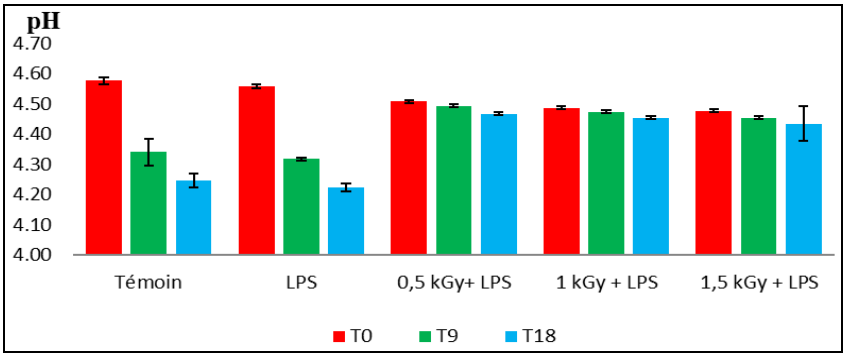

Fig 1: Effect of ebeam irradiation on the pH of fresh cheese (Control; LPS; 0.5kGy +LPS; 1 kGy+LPS; 1.5kGy+LPS)

Effect of Hurdle technology on acidity

The acidity developed in cheese results from the transformation of lactose into lactic acid. It is measured by titration (Gadi *et al*., 2020). The low titratable acidity values recorded in our results reflect weak lactic fermentation in the cheese samples, which depends on the casein, mineral salt and ion content. It also depends on the hygienic conditions during milking, the total microbial flora and its metabolic activity and the handling of the milk (Gadi *et al*., 2020).

Fig 2 Shows the effect of cheese acidity at t_0, t_9 and t_{18} of storage at 4±1°C after a combination of ebeam treatment with 0.5; 1 and 1.5 kGy and LPS activation compared to a control and a sample activated only with LPS. We can see that there was a significant increase in acidity in the cheeses that had undergone electron beam irradiation combined with LPS compared with the other control cheeses and those activated by LPS only at t_0.

However, the increase in acidity in cheeses irradiated with electron beams combined with LPS compared with control cheeses and cheeses irradiated with LPS alone was not significant at t_9 and t_{18}.

This increase can be explained by the deacidifying effect of certain germs such as psychotropics and moulds and the resulting degradation or inhibition of the microbial flora and in particular the lactic acid bacteria responsible for the acidification of the cheese (Leksir, 2018).

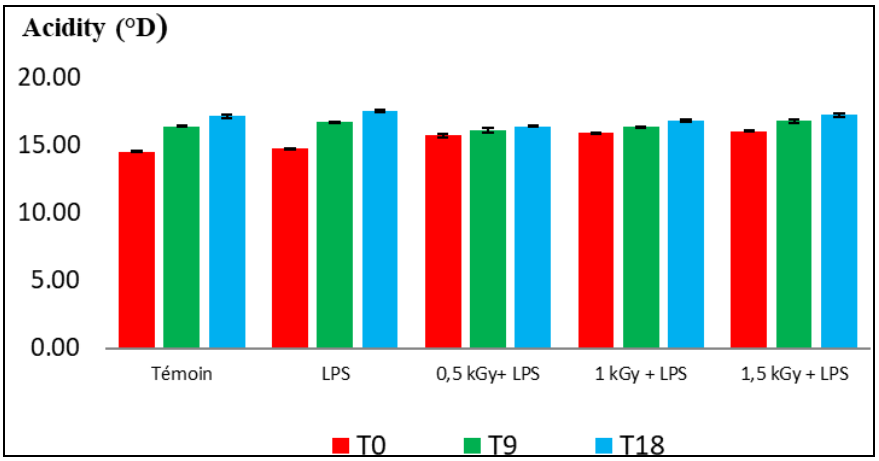

Fig 2: Effect of ebeam irradiation on titratable acidity of fresh cheese (Control ; LPS ; 0.5kGy +LPS ; 1 kGy+LPS; 1.5kGy+LPS)

Effect of Hurdle technology on total dry extract

The level of dry extract varies from one type of cheese to another. This difference is due to the use of salt and the draining time. The cheese's high dry matter content gives it a relatively firm consistency.

Fig 3 shows the effect on the total dry extract of the cheese at t_0, t_9 and t_{18} of storage at 4±1°C after a combination of ebeam treatment with 0.5, 1 and 1.5 kGy and LPS activation compared with a control and a sample activated only with LPS.

Graphical analysis shows an increase in dry extract content as a function of dose, from a minimum of 35.73 g/kg (Control) to a maximum of 36.93 g/kg (1.5 kGy).

This increase was significant at t_0, t_9 and t_{18} and could be due to the phenomenon of radiolysis of the water, which increased the percentage of dry extract.

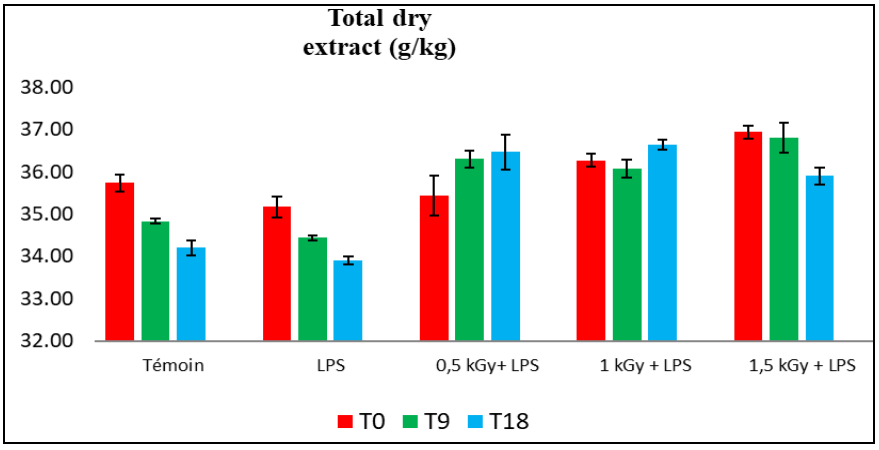

Fig 3: Effect of ebeam irradiation on the total dry extract of fresh cheese (Control; LPS; 0.5kGy +LPS; 1 kGy + LPS; 1.5kGy+LPS)

Effect of Hurdle technology on fat content

Fat content and dry matter are very important in cheese production because milk lipids are characterised by the presence of relatively short-chain fatty acids which can be absorbed by a simpler mechanism than long-chain fatty acids. Milk fat plays an essential role in the development of both the taste and texture of cheese (Vignola, 2002) [19].

Cheese texture depends on its fat content. In fact, the water content and the proportions of long polysaturated fatty acids in the milk determine the texture of the dough: extra hard,

semi-soft, soft, and so on. For example, with more than 60% fat and less than 51% water, you get an extra-hard cheese. Too high a fat content can lead to problems with draining and coagulation (Vignola, 2002) [19].

Fig 4 shows the variation in the fat content of cheeses treated with the first combination and control cheeses over a storage period of 18 days at 4±1°C.

The highest dose 1.5 kGy combined with LPS activation had a significant effect on fat content compared to all other samples throughout the storage period.

However, there was a slight increase in fat content as a function of dose, but it did not exceed 2% in the control cheeses and those activated by LPS and those irradiated with 0.5 and 1 kGy combined with LPS at t_0.

On the other hand, all the other samples showed a significant increase compared with the controls and those activated by LPS at t_9 and t_{18}.

This increase could be due to oxidation of the lipids by the irradiation process, producing OH radicals formed mainly as a result of radiolysis of water.

This was approved by Hyun *et al*, (2010) who showed that electron beam irradiation induced higher values of thiobarbituric acid reactive substances (TBARS) in samples irradiated at 5 kGy than in other samples.

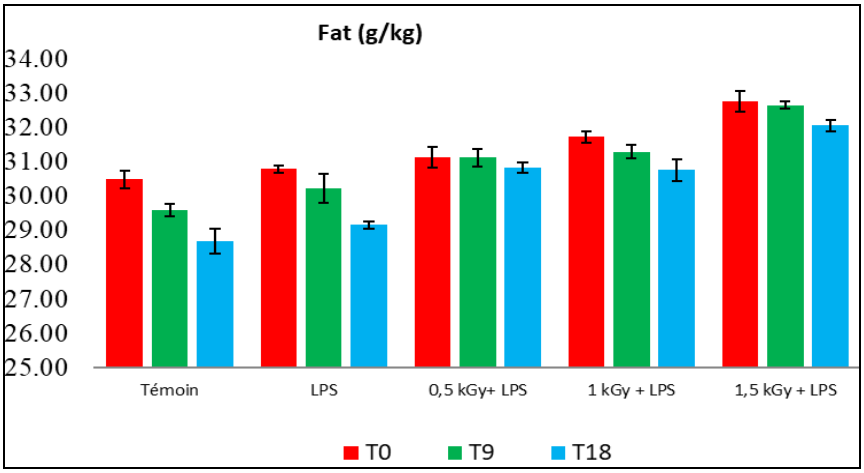

Fig 4 : effect of ebeam irradiation on the fat content of fresh cheese (Control; LPS; 0.5kGy +LPS; 1 kGy+LPS; 1.5kGy+LPS)

Effect of Hurdle technology on defatted dry extract

Figure 5 shows the variation in fat-free dry matter of treated and untreated cheeses stored at 4±1°C. It can be seen that the defatted dry extract values increased in proportion to the technique applied, rising from 5.40, 4.53 and 4.17 g/kg at t_0.

The three doses applied did not have a significant effect ($p>0.05$) on the fat-free dry extract of the cheeses compared with the control cheeses and those activated by LPS throughout the shelf life.

However, there was a significant variation in the defatted dry matter of cheeses irradiated with the highest dose (1.5 kGy) combined with LPS compared with control cheeses and those activated by LPS throughout t9.

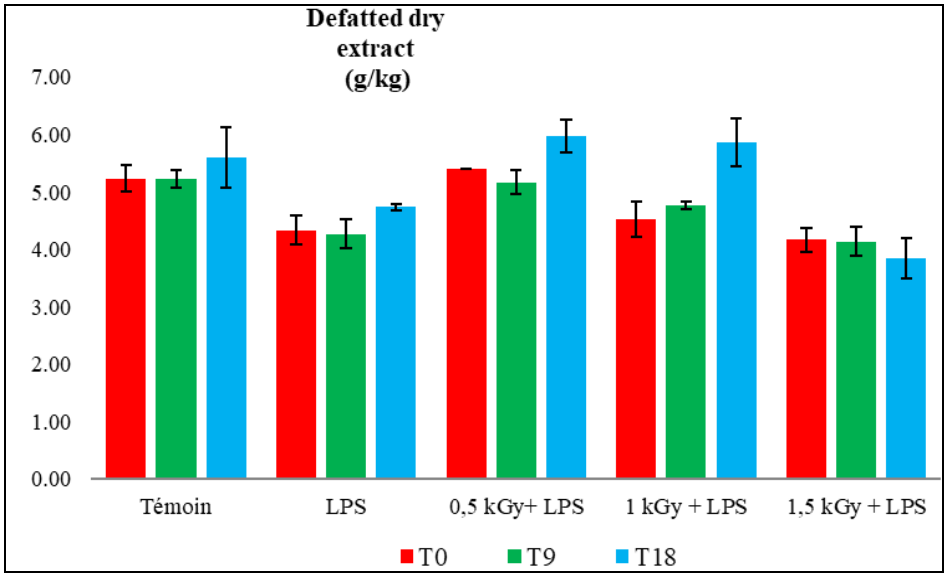

Fig 5: Effect of ebeam irradiation on the defatted dry extract of fresh cheese (Control; LPS; 0.5kGy +LPS; 1 kGy+LPS; 1.5kGy+LPS)

Effect of Hurdle technology on humidity

For the moisture parameter, if the values are outside the standards, there is a risk of having a fairly hard dough (if the moisture content is less than 58%), or a fairly moist, and therefore fragile, dough if the moisture content% is greater than 60%. The TSE content is linked to the moisture

content%, so outside the range given in table (40-42), there is a risk of varying the moisture content% and therefore ultimately contributing to the hardness or brittleness of the dough.

Fig 6 shows that the control cheese samples had an average moisture content of 64.27% and the LPS-activated cheese had a value of 64.83%, while the irradiated cheese samples had values of 62.47%, 61.83% and 60.03% respectively at t_0.

In fact, there was a significant difference between the control and irradiated groups combined with LPS, since there was a significant decrease in the cheeses with the highest dose combined with LPS compared with all the other samples at t_0.

Moreover, the other two doses combined with LPS had a significant effect compared with the controls and those activated by LPS. On the other hand, no significant variation in humidity was observed in the LPS-activated cheese compared with the controls at t_0.

On the other hand, there was a significant difference between the cheeses that had been subjected to the different doses with LPS in terms of moisture compared to the controls and those activated only by LPS, and no significant difference between the cheeses activated by LPS and the controls at t_9 and t_{18}. Thus the moisture content of the irradiated cheese samples was significantly lower than that of the controls and those activated only by LPS.

This decrease can be attributed to the effect of gamma irradiation on the capacity of cheese proteins to retain water. Almost similar results were reported by Gosh *et al*, (1999).

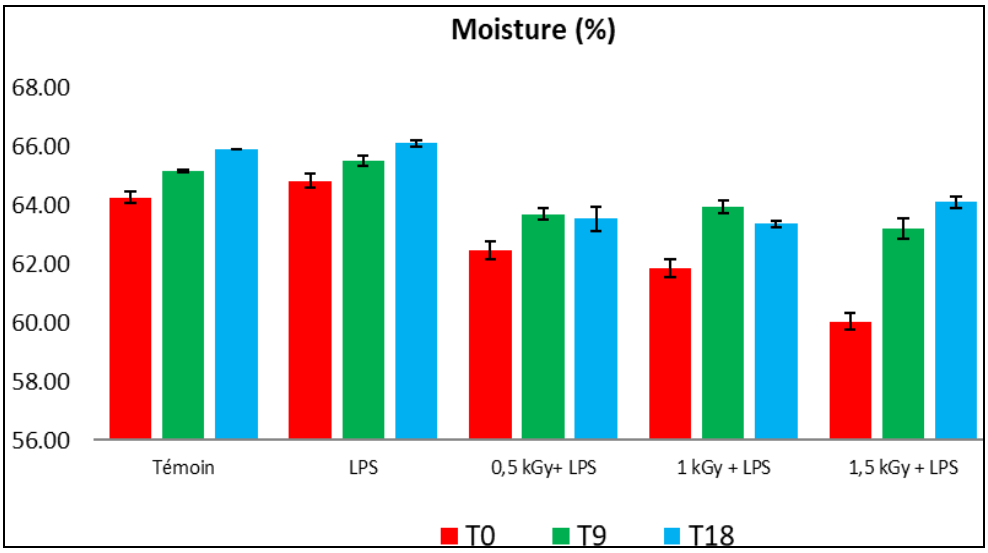

Fig 6 : Effect of ebeam irradiation on the moisture of fresh cheese (Control; LPS; 0.5 kGy +LPS;1 kGy+LPS; 1.5kGy+LPS)

Rheological study

Texture is an essential factor in consumer acceptance of a product. The term 'firmness' is commonly used to describe a parameter assessed by means of empirical mechanical tests and considered as an attribute that must be maintained during storage and processing.

Table 1 shows the variation in firmness of treated and untreated cheeses stored at 4±1°C.

It can be seen that the average firmness value of the cheeses decreases directly following irradiation and over time.

In fact, non-irradiated cheeses went from 0.28 to 0.2 N after 18 days of refrigerated storage.

For cheeses activated only by LPS, firmness fell from 0.277 to 0.21 N after 18 days of refrigerated storage.

As for the cheeses treated with the low dose combined with LPS, their firmness fell from 0.27 to 0.21 N. For the medium dose (1 kGy) combined with LPS, the average firmness value rose from 0.27 to 0.20 N.

For the 1.5 kGy dose with LPS, a decrease was observed, reaching 0.26 N after 18 days of conservation.

This decrease in texture over time could be due to the migration of calcium in response to the pH gradient and the activities of fungal enzymes and rennet. Several authors, Spinnler *et al*, (2004) [18] have reported that the change in texture is due to this phenomenon, as calcium phosphate precipitates at the generally high pH of cheese rinds. Calcium from the core then migrates to the surface to re establish the equilibrium, causing the micelles to destabilise and the cheese to soften as a result McSweeney, (2004) [11]. The changes observed in the texture of the untreated and treated samples could be linked to this phenomenon.

However, the three doses applied in combination with LPS did not have a significant effect on cheese firmness at t_0, t_9 and t_{18}.

Table 1: Effect of ebeam irradiation on the firmness of fresh cheese (Control ; LPS ; 0.5kGy +LPS ; 1 kGy+LPS; 1.5kGy+LPS)

Time Doses	T_0	T_9	T_{18}
Control	0,28±0,0	0,247±0,005	0,2±0,01
LPS	0,277±0,01	0,243±0,01	0,21±0,00
0,5kGy	0,273±0,01	0,24± 0,00	0,207±0,01
1 kGy	0,27±0,0	0,237± 0,00	0,2±0,0
1,5 kGy	0,265±0,01	0,233± 0,01	0,187±0,01

Microbiological quality

The results of the evolution of the microbial flora of the cheeses at (t_0) and after 9 and 18 days of storage at 4±1°C.

The germs sought and counted in our work are considered to be indicators of the overall quality of the finished product and reflect compliance or non-compliance with good hygiene practices (Gadi *et al*., 2020).

Table 2: Effect of combining ebeam with LPS activation on microbiological quality

	Total coliforms (UFC/g)	Total mesophilic aerobes (UFC/g)	Yeasts and molds (UFC/g)
Témoin	t_0 : 0,53±0,09	t_0 : 3,00±0,09	t_0 : 1,48±0,00
	t_9 : 1,41±0,03	t_9 : 4,34±0,01	t_9 : 3,88±0,02
	t_{18} : 2,87±0,03	t_{18} : 4,52±0,02	t_{18} : 3,97±0,01
LPS	t_0 : 0,34±0,11	t_0 : 2,93±0,02	t_0 : 1,52±0,04
	t_9 : 1,34±0,03	t_9 : 4,27±0,01	t_9 : 3,85±0,01
	t_{18} : 2,87±0,00	t_{18} : 4,45±0,01	t_{18} : 3,95±0,02
LPS + 0,5kGy	t_0 : 0,00±0,00	t_0 : 0,59±0,05	t_0 : 1,04±0,07
	t_9 : 0,95±0,08	t_9 : 4,19±0,01	t_9 : 2,33±0,00
	t_{18} : 1,13±0,07	t_{18} : 4,27±0,02	t_{18} : 3,69±0,02
LPS + 1 kGy	t_0 : 0,00±0,00	t_0 : 0,07±0,13	t_0 : 0,88±0,1
	t_9 : 0,81±0,1	t_9 : 3,99±0,01	t_9 : 2,21±0,02
	t_{18} : 0,95±0,03	t_{18} : 4,18±0,01	t_{18} : 2,45±0,03
LPS + 1,5 kGy	t_0 : 0,00±0,00	t_0 :0,00±0,00	t_0 : 0,62±0,17
	t_9 : 0,46±0,15	t_9 : 1,39±0,03	t_9 : 1,68±0,14
	t_{18} : 0,78±0,17	t_{18} : 1,47±0,02	t_{18} : 2,10±0,00

Total coliforms

In fact, the number of total germs reflects the level of hygiene of the cheese samples.

Table 2 shows a total absence of total coliforms immediately after treatment (t_0) with all the doses combined with LPS. Even with LPS activation alone, there was a reduction in proliferation compared with the control.

For products treated with different doses combined with LPS, we can see that the total coliforms were reactivated, hence their increase over time. This increase in the irradiated products was still less than in the control and LPS-activated cheeses.

The numbers in all the groups irradiated with LPS decreased with increasing irradiation dose.

In fact, at t_0 there was a significant decrease at all irradiation doses with electron beams combined with LPS compared with the control and LPS-activated cheeses, and LPS activation significantly reduced the evolution of total coliforms.

On the other hand, at t_9 there was a significant decrease in the evolution of total coliforms in the cheese irradiated with the highest dose, 1.5 kGy combined with LPS, compared with the control and activated by LPS and the other two doses. It was also noted that irradiation of the cheeses with 0.5 and 1 kGy combined with LPS resulted in a significant decrease in the evolution of total coliforms compared with the control and LPS.

However, there was no significant increase in total coliforms in the LPS-activated cheese and the control.

Similarly, at t_{18}, the same results as those obtained at t_9 were observed, with the exception that there was no longer a significant decrease in the evolution of these germs in the cheese irradiated with the highest dose and that at 1 kGy.

In fact, the 1.5 kGy dose combined with LPS was the best of the three tested against total coliforms, as it allowed total inhibition at t_0 and caused the greatest reduction during storage.

Similar results were reported by Aly *et al*, (2012) who showed that gamma irradiation at high doses which are 1;3 and 5 kGy decreased total coliforms.

This was probably due to the effect of the energy produced from the irradiation breaking the DNA bonds. Some bacteria can repair DNA strand damage and resist the effect of irradiation. The effectiveness of the process depends on the organism's sensitivity to irradiation, the rate at which it can repair damaged DNA, and in particular the amount of DNA in the target organism. It also depends on pH, temperature, water activity (Aw), and the nature of the radiation used in the process (Molins *et al*., 2001) [12].

The results obtained stated that the use of the hurdle technique significantly reduced the growth of total coliforms at low doses.

Mesophilic aerobes

The number of total aerobic mesophilic flora in cheese can cause premature swelling and the production of enterotoxins in cheese (Seifu *et al*., 2004) [17].

The application of ebeam and the activation of LPS made it possible to reduce mesophilic aerobes directly after treatment (t_0) until a total absence of mesophilic aerobes for the combination of LPS with the highest dose of 1.5 kGy, as shown in Table 2.

Moreover, this combination of LPS with the highest dose (1.5 kGy) at t0 resulted in a significant reduction compared to that of the control cheeses and those activated by LPS and those with a dose of 0.5 kGy combined with LPS.

With an initial control of 3.0 log CFU/g, there was a slight but non-significant reduction ($p>0.05$) for the cheese activated only by LPS at 2.93 log CFU/g.

In fact, the reduction in this flora in cheeses made from milk preserved by activation of the LPS system suggests that activation of this system in the milk before cheese manufacture could be of great practical importance (Seifu *et al*., 2004) [17].

Moreover, Seifu *et al*, (2004) [17], found that preservation of cheese milk by the LPS system can be used to improve microbiological quality. At t_9 and t_{18}, there was a significant difference in all samples.

However, during storage, aerobic mesophilic bacteria increased. In fact, after one week of refrigeration, there was an increase in aerobic mesophiles compared to t_0. This increase noted at t_9 can be explained as follows: the rays damaged and lysed the cells of the microorganisms at t_0 and over time, and thanks to their biological systems and resistances, this lysis was repaired.

Our results are consistent with those of Hyun *et al*, (2010) who reported that at t_0 the mesophilic load was absent at the highest dose of 3kGy.

Irradiation of 1 and 2 kGy reduced the microbial load by approximately 1 or 2 decimal units of the original microbial load.

The use of the combination of electron beam irradiation at low doses produced similar results to those obtained by the use of electron beam irradiation at high doses.

Yeasts and moulds

Yeasts and moulds in cheese are considered to be spoilage organisms leading to flavour and texture deterioration, including softening and discolouration (Aly *et al*., 2012).

For yeasts and moulds (table 2), there was a significant decrease in the microbial load following the combination of ebeam with LPS.

In fact, the lowest dose and the average doses of 0.5 and 1kGy combined with LPS resulted in an average value for yeasts and moulds (1.45 and 0.81 log CFU/g respectively) compared with the control, which was initially 2.34 log CFU/g and 2.31 log CFU/g for those activated only by LPS, and almost zero with the highest dose combined with LPS.

No significant difference in the number of moulds was observed between the control and the LPS-activated cheeses at t0.

Moreover, these results are approved by Seifu *et al*, (2004) [17] who demonstrated that LPS activation has no significant effect on the evolution of yeasts and moulds.

During storage at 4±1°C, the yeast and mould levels of untreated (non-irradiated) raspberries increased over time to 3.97 compared with those treated only with LPS, which had an average value of 3.95 log CFU/g at t_{18}.

Similarly, for products irradiated and combined with LPS, an increase in fungal growth was observed over time, but this increase remained significantly lower than for the control and that activated only by LPS at t_9 and t_{18}.

Similar results were reported by Aly *et al*, (2012) who showed that gamma irradiation at the highest dose of 5 kGy reduced the proliferation of these microorganisms. The doses used by these authors were 1, 3 and 5 kGy.

Sensory analysis

The sensory analysis, hedonic test, was carried out directly after treatment at t_0, t_9 and t_{18} days of storage at 4°C on control cheeses, activated by LPS, having undergone irradiation at 0.5, 1 and 1.5 kGy combined with LPS. We chose to carry out these analyses throughout the 18-day storage period on the basis of the cheese quality assessed.

The characterisation of the sensory properties of the cheeses was analysed using the radar diagram constructed with the scores obtained for the different parameters evaluated by the panellists.

On the basis of the deterioration index reported, if the overall acceptability score was less than 5, the product would be considered a putrid food (Lainez *et al*., 2008) [10].

The figures represent the results of the sensory analysis of the cheeses after treatment (t_0), 9 days after irradiation (t_9), 18 days after irradiation (t_{18}) stored at 4°C.

The "aroma" descriptor for the cheeses was scored as follows: the highest score was awarded for cheeses activated only by LPS at t_0, scored at 5.58 out of 9, but decreased to 3.83 towards the end of storage at t_{18}.

On the other hand, the lowest score was awarded to cheese irradiated with electron beams in combination with LPS, which totalled 4.83 out of 9 and fell over time to 4.17 at t_{18}.

For the other descriptors such as salty taste and acid taste, the lowest scores were attributed to the control cheeses, which scored 5.33 and 5.08 out of 9 respectively at t0 and decreased over time to 5.00 and 4.67 out of 9 at t_9. The panel gave a score of 4.50 and 3.75 over 18 days of storage.

In fact, the descriptors studied, such as colour, acidity, texture and odour, and linking them to physicochemical parameters, will be detailed below.

For the "texture" descriptor, the scores attributed by our panel to the 0.5, 1 and 1.5 kGy doses combined with LPS had no significant effect ($p>0.05$) on cheese texture. This is confirmed by the analytical test measuring the firmness of the cheeses, which found that the different doses combined with LPS had no significant effect compared to the controls or to the cheeses activated only by LPS. However, the panel found that texture deteriorated significantly over time.

For the descriptor "colour", the scores attributed by our panel to doses of 0.5, 1 and 1.5 kGy combined with LPS had no significant effect ($p>0.05$), but we note that time significantly affected colour at t_{18} only, whereas there was no significant difference between t0 and t_9.

Concerning the odour descriptor rated by the panel at t_0, t_9 and t_{18}. The scores given are not significant ($p>0.05$). In fact, there were no significant differences between all the samples. On the other hand, we note that time has a significant effect throughout the shelf life. These two descriptors, colour and odour, were highly correlated ($p<0.01$).

On the other hand, there was no significant difference in overall acceptability between all the samples, although it was noted that shelf life affected overall acceptability significantly at t_{18} only, whereas there was no significant difference between t_0 and t_9. Moreover, overall acceptability was strongly correlated with colour, odour and texture ($p<0.01$).

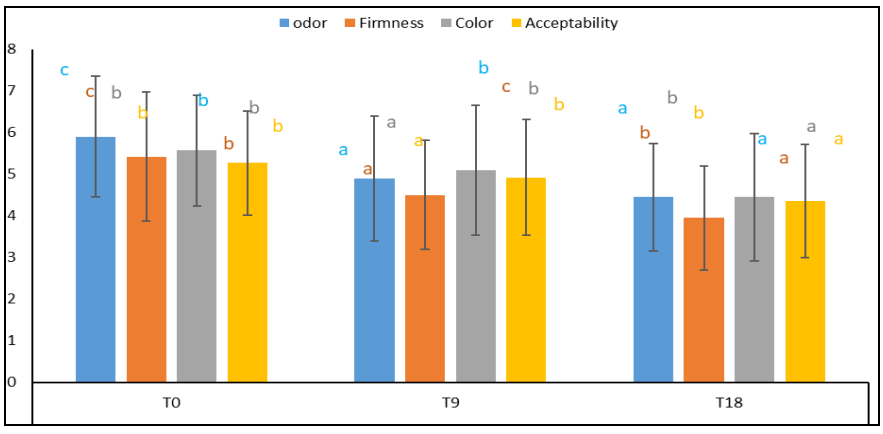

Fig 7 : Effect of ebeam irradiation with LPS on the organoleptic properties of fresh cheese.

Effect of hurdle technologies on the evolution of the the shelf life of cheese

Estimation of the use-by date using the accelerated aging test

The spoilage kinetics of a foodstuff is a representation of the deterioration of a parameter A as a function of time and temperature. These kinetics are generally of order zero or of order one.

The determination of the order of the spoilage reaction is obtained by comparing the coefficient of determination R2 of the linear regression of the three kinetic models related to a quality criterion A by drawing the graphs:

Order zero: A = f(t)

Order 1: Ln (A) = f(t)

Order 2: (1/H) = f(t)

Determination of the use-by date by monitoring the evolution of yeasts and molds, indeed the microbial limit of acceptability was estimated by fitting the experimental data to the Gompertz equation modified by Corbo (Zantar *et al*., 2013) [21]

A concentration ≥ 105 CFU / g of yeasts and molds marks the end of the useful life of fresh cheese. This level of contamination corresponds to the appearance of defects, abnormal colors and odors (Zantar *et al*., 2013) [21]

We observed that the combination prolonged the shelf life of fresh cheese compared with control cheeses and those activated only by LPS.

This shows that irradiation has a significant bacteriostatic or bacteriocidal effect. It also confirms the synergistic effect of ebeam and LPS, since we recorded an increase in the shelf life to 6.82, 10.92 and 16.93 days respectively for 0.5, 1 and 1.5 kGy combined with activation of the lactoperoxidase system.

Purpose and objectives

The aim of the project is to meet consumer requirements in terms of food safety and quality while improving the shelf life of cheese. This study aims to compare the effect of combining electron beam irradiation with lactoperoxidase activation as a decontamination tool.

To this end, microbiological, physico-chemical (titratable acidity, pH, total dry extract, fat, non-fat dry extract, moisture and firmness) and organoleptic analyses were carried out during storage of the cheeses at +4±1ºC for 18 days, and the shelf life of the products was determined.

Conclusion

Today, one of the main challenges facing industry and the economy is to meet the needs of a growing world population while preserving the environment.

The aim of the agri-food industries is to introduce a rational use of renewable raw materials that exploit the complementary nature of the food and non-food sectors. This study was targeting the industrial market in order to encourage it and to replace the conventional techniques with more ecological, innovative and sustainable ones.

We were able to demonstrate that the storage combination had no significant effect on texture throughout the duration of refrigerated storage. On the other hand, the combination had a significant impactt on all the physico-chemical parameters (pH, titratable acidity, total dry extract, fat, defatted dry extract and moisture) throughout the shelf life.

On the other hand, the panel did not detect any significant effect on the descriptors (colour, odour, texture and overall acceptability) for the the combination throughout the shelf life.

The study of the microbiological quality of the cheese showed that irradiation with ebeam combined with LPS had a significant effect on the microbiological quality (total coliforms, mesophilic aerobes, yeasts and moulds) compared with control cheeses and cheeses activated only by LPS.

In addition, the determination of the shelf life showed that the use of these combination resulted in its extension of by 3.63 days, compared with 3.09 days for the LPS cheese alone.

In fact the combination, low-dose electron beam irradiation with LPS extended the shelf life of the cheese to 6.82 days, compared with 10.92 and 16.93 days respectively for 1 and 1.5 kGy combined with activation of the lactoperoxidase system.

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