SALES UNIVERSITY OF SALERNO

Research laboratories:

*Plants & Processes - DIFARMA*
*Microbiology - DIFARMA*

Contract for consultancy services concerning:

***Protocols for the LED irradiation of food to extend shelf life***

# TECHNICAL-SCIENTIFIC REPORT OF THE ACTIVITIES CARRIED OUT

*Meat substrates*: Hamburger*

*Contents:*

Preparation of irradiation areas

Monitoring sensory and textural properties

Microbiological monitoring

* delivered on February 22, 2019


**Summary technical-scientific opinion**

*The LED light of the Biovitae® device showed bacteriostatic capacity when irradiated on meat substrates such as the hamburgers in question, in all the operating conditions examined and detailed in this report. Its application is particularly suitable for maintaining the microbiological safety properties if the substrates, for various reasons, are exposed to ambient conditions for prolonged times. Its application, again due to the bacteriostatic action, is certainly favorable even in a refrigerated environment.*
*The irradiation of the examined substrates involves a global moderate acceleration of the variations of the sensory characteristics (color, texture) with respect to the non-irradiated samples, placed in the same observation conditions, used as control.*



*Prof. Ing. Anna Angela Barba*


**ABSTRACT**

Object of the study:

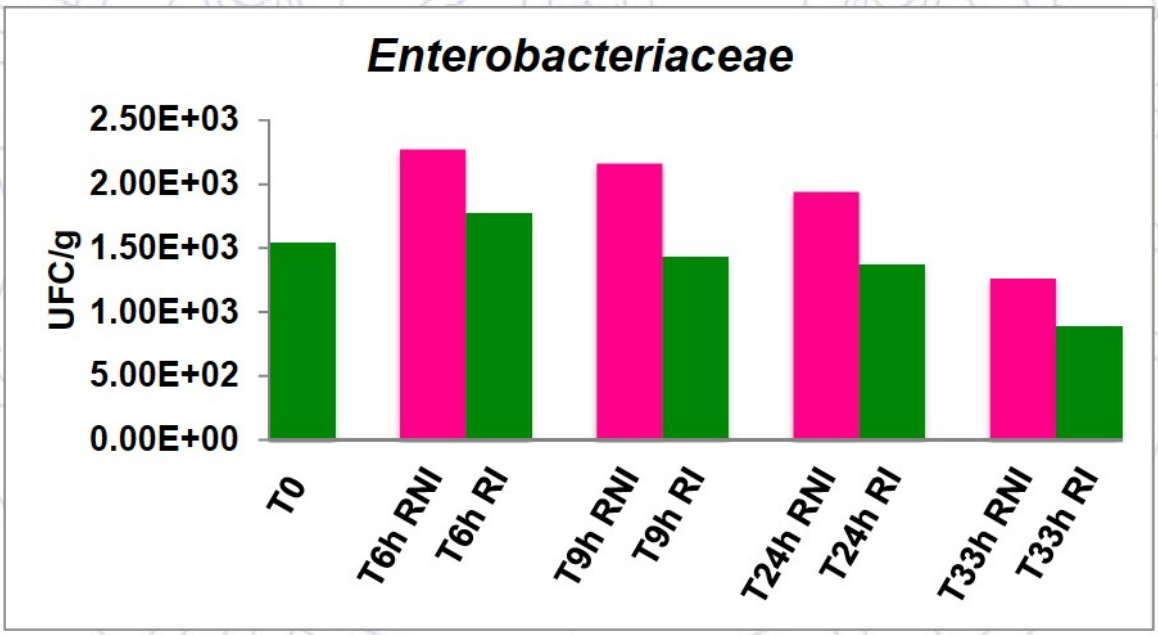
The substrates under study were hamburgers.
The substrates delivered pending the implementation of the monitoring protocols were stored in refrigerator at about 4 ° C.
The monitoring parameters that appeared most significant for the investigation are:
- sensory properties: free water loss (residual moisture), texture, color.
- microbiological profile: total bacterial load of the product, possible search for specific bacterial contaminants

Exposure protocols and monitoring timing planning
The irradiation and therefore monitoring activities were carried out in a 33-hour time frame (24 hours, plus 9 hours) such as to include more work shifts in industrial kitchens where hamburgers are handled (interruption of the cold chain, opening of packaging, preparations ).
The irradiation protocols have been defined by arranging different areas of exposure.
For the irradiation, two Biovitae® lamps were placed on a rigid support so as to illuminate a work area of approximately 0.20 m2.

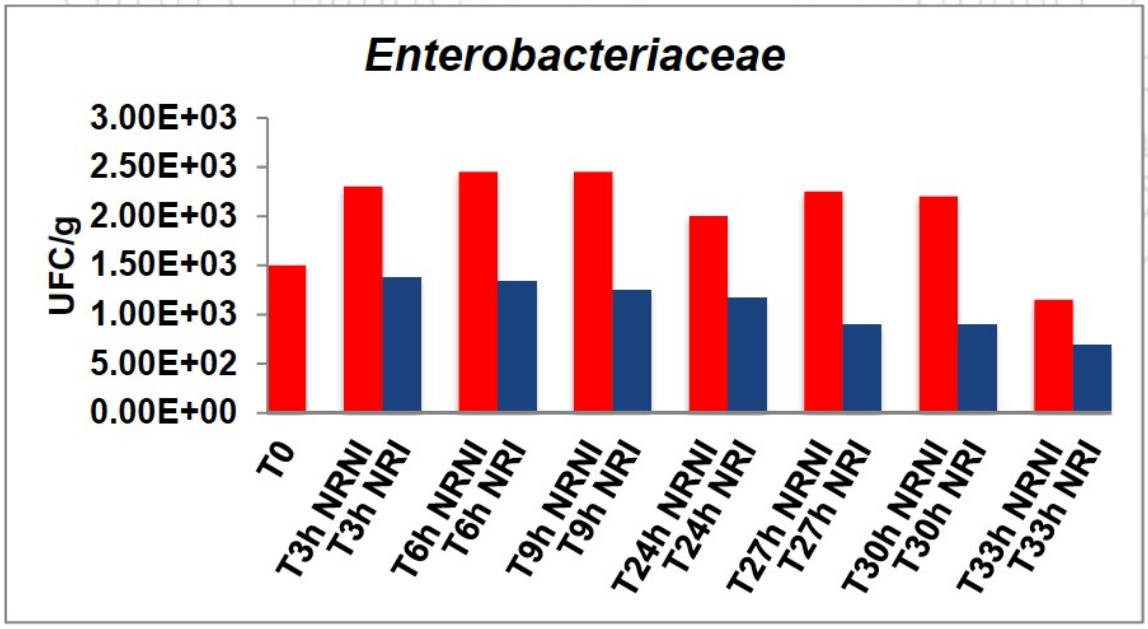
Irradiation and control protocol for non-irradiated samples
The management of the monitoring activity was organized in 8 simulating study lines, possible real operational scenarios. Among these, some exhibits more penalizing operating conditions such as non-refrigerated environment and absence of gloves during substrate handling operations. In detail, the study lines are organized as follows:

Test n. 1 substrates open at time 0 h, exposed for an appropriate time interval under conditions environmental with periodic manipulation;
Test n. 2 substrates open at time 0 h, exposed for an appropriate time interval under environmental conditions, irradiated with periodic manipulation;
Test n. 3 substrates open at time 0 h, exposed for a suitable time interval under conditions refrigerated with periodic handling;
Test n.4 substrates open at time 0 h periodic manipulation;
Test n. 5 substrates open at time 0 h, exposed for an appropriate time interval under conditions environmental with periodic handling without gloves;
Test n. 6 substrates open at time 0 h, exposed for an appropriate time interval under environmental conditions, irradiated with periodic manipulation without gloves;
Test n. 7 substrates open at time 0 h, exposed for an appropriate time interval under conditions refrigerated with periodic handling without gloves;
Test n. 8 substrates open at time 0 h, exposed for an appropriate time interval under conditions refrigerated, irradiated with periodic handling without gloves.

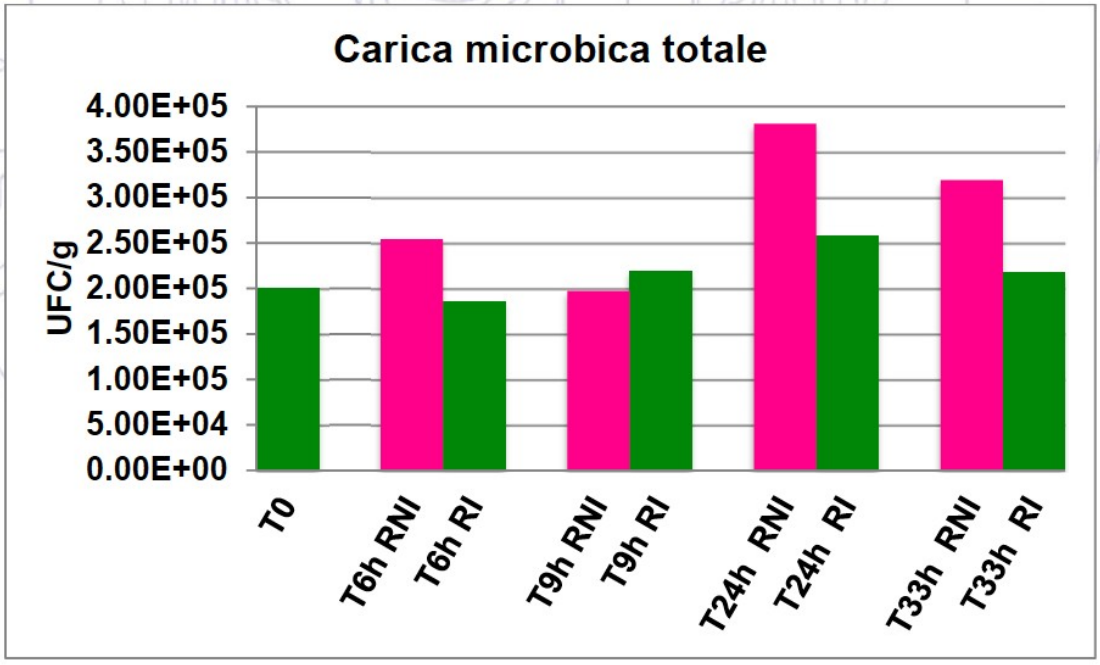
Tables



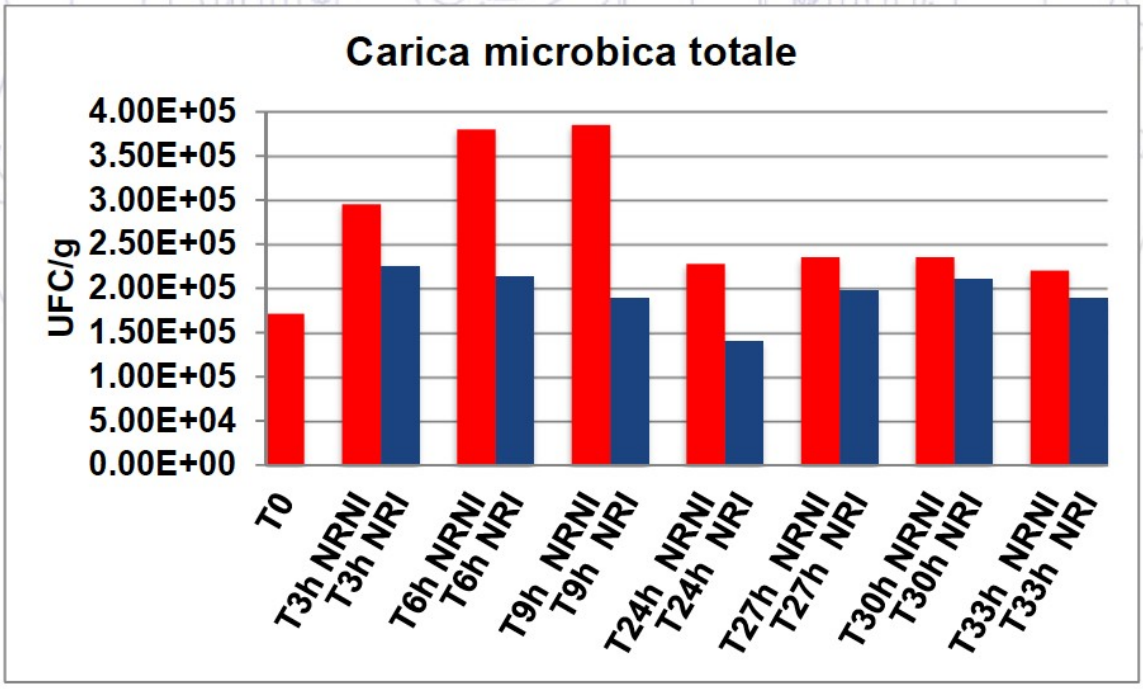

Enterobacteriaceae counts in different samplings carried out over time for a total of 33 hours starting from refrigerated substrates with periodic manipulation without gloves and without irradiation (Test 7, RNI), or with irradiation (Test 8, RI).




Enterobacteriaceae counts in different samplings carried out over time for a total of 33 hours starting from conserved substrates at room temperatures with periodic manipulation without gloves and without irradiation (test 5, NRNI), or with irradiation (test 6, NRI).




Total microbial count in different samplings carried out over time for a total of 33 hours starting from substrates refrigerated with periodic manipulation without gloves and without irradiation (test 7, RNI), or with irradiation (test 8, RI).




Total microbial count in different samples carried out over time for a total of 33 hours starting from substrates stored at room temperature with periodic manipulation without gloves and without irradiation (test 5, NRNI), or with irradiation (test 6, NRI).

conclusions: the Biovitae® device has shown itself capable of containing the bacterial load of the food so as to preserve its safety characteristics even if for long periods (33h) the cold chain has been interrupted. It has also clearly shown a capacity to reduce the bacterial load even in storage conditions in the refrigerator.

# Summary

# FOREWORD, OBJECTIVES AND STRUCTURE OF THE TECHNICAL-SCIENTIFIC REPORT

In industrial kitchens, strict hygiene protocols are respected in order to preserve the hygienic-sanitary characteristics of food products. Bacterial contaminations are however unavoidable, and therefore when the conservation history of a semi-finished product (cold chain, vacuum or controlled atmosphere packaging) is rapidly interrupted, a deterioration path begins which makes the semi-finished product unusable within a predetermined period . The economic damage is evident, and if, in order to minimize the economic damage, the contaminated semi-finished product was used equally, even more serious damages could be encountered.

Since the main cause of degradation is bacterial contamination, the most reasonable approach to decrease the contamination kinetics is to control the bacterial load that comes into contact with the food being processed. Obviously one must disregard the use of chemical substances (which can contaminate food), the use of physical means which can modify the organoleptic and nutritional characteristics (temperature), or which can interfere with the handling activities (UV rays). A possible solution is the use of low wavelength LED light, which has a proven bactericidal power, as in the case of the Biovitae® device.


The aim of this research is, therefore, the application of irradiation protocols with LED light of the Biovitae® device to food semi-finished products (meat substrates), with consequent monitoring of sensory and microbiological properties, in order to test the maintenance period of the safety properties microbiological (shelf life over time) after the interruption of primary conservation methods (cold chain, vacuum).

This report reports the study carried out on hamburgers (a substrate made of minced meat). It is divided into two different sections. In the first, the results and conclusions of the monitoring of sensory, textural and microbiological properties of the irradiated substrates are presented and discussed (as control, substrates placed in the same operating conditions but not irradiated were used), preceded by introductory paragraphs describing the study approach. (irradiation configuration, study lines). The second section summarizes the methods applied.

# SECTION 1 RESULTS AND DISCUSSION

## 1.1 Substrates, storage conditions, monitoring parameters

The substrates studied were burgers. They were supplied by the customer in refrigerated conditions, individually vacuum packed.

The substrates delivered pending the implementation of the monitoring protocols were stored in a refrigerator at about 4 ° C.

The monitoring parameters that appeared most significant for the investigation are:

- sensory properties: loss of free water (residual moisture), texture, color.
- microbiological profile: total bacterial load of the product, possible search for specific bacterial contaminants.

## 1.2 Exposure protocols and monitoring timing planning

The irradiation and therefore monitoring activities were carried out in a 33-hour timespan (24 hours plus 9 hours) such as to include more work shifts in industrial kitchens where the handling of hamburgers takes place (interruption of the cold chain, opening of packaging, preparations ).

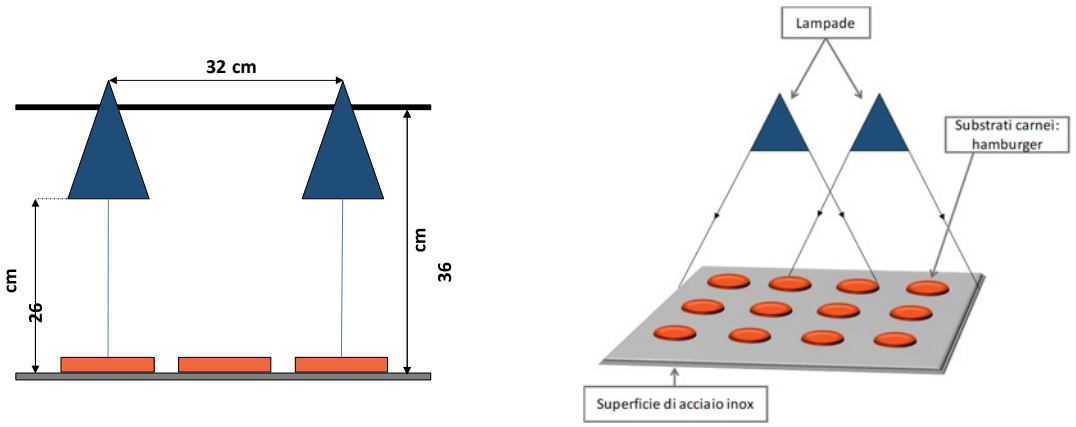
The irradiation protocols have been defined by setting up different areas of exposure (described immediately below).

The sampling time for monitoring activities was defined in relation to the operating conditions applied.

### 1.2.1 Areas of exposure and configuration of radiation loads

Once released, the substrate samples were placed on a stainless steel surface washed with appropriate detergents at the beginning of the monitoring activities.

For radiation two Biovitae® lamps[1]they have been positioned on a rigid support in order to illuminate a work area of approximately 0.20 m2 (an area that allows good lighting of more than a dozen medium-large hamburgers arranged in a single layer). The layout of the LED devices is shown in Figure 1:




*Irradiation apparatus used for activities experimental*

*LED lamps and position of radiated loads (in the further details are given in the following paragraphs)*

***Figure 1. Diagram of the arrangement of LED devices; configuration of lamps - irradiated loads.***

The radiation pattern (load configuration) has been kept unchanged for all the study lines, coding the relative load / LED source positions to try to observe the effect of the view factor.

[1] Selected and delivered by the client.

For the monitoring activities, it was decided to equip 3 different work areas to simulate the most probable working environment conditions:

- area of exposure and handling at ambient conditions without irradiation of the samples (Figure 2 a);
- area of exposure and handling at ambient conditions with irradiation of the samples (Figure 2 b);
- display area and handling under refrigerated conditions without and with irradiation of the samples (positioning of the entire irradiation apparatus in a thermostat, Figure 3 a and 3 b).

The first 2 areas of exposure and handling are in an open environment (research laboratory) hosting the passage of 3 - 4 different people during the monitoring period. The third display and handling area is in a confined environment (thermostat).

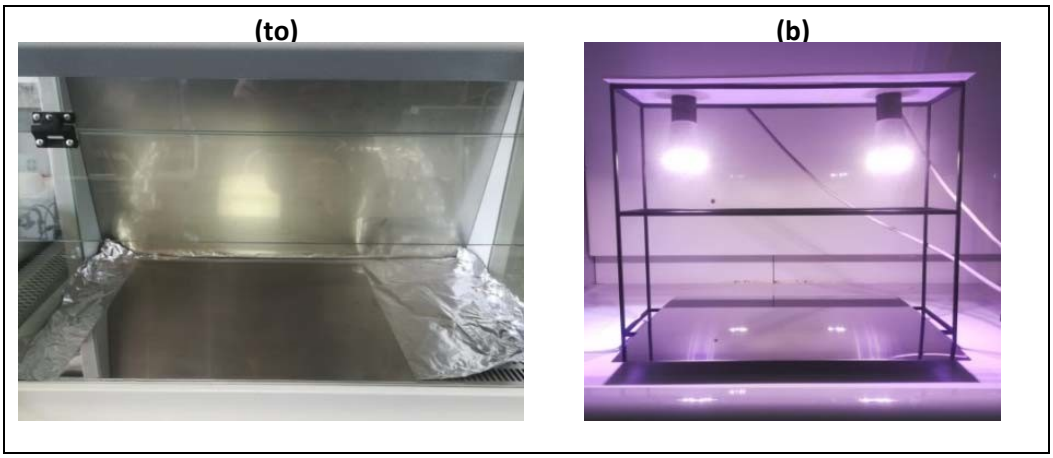



***Figure 2. (a) area of exposure and handling at ambient conditions without irradiation of the samples; (b) area of exposure and handling at ambient conditions with irradiation of the samples.***

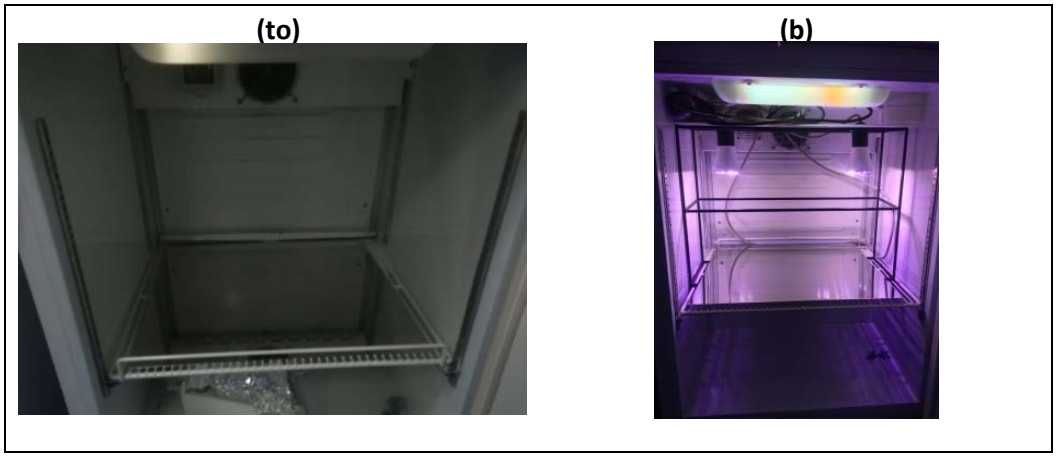



***Figure 3. (a) area of exposure and handling under refrigerated conditions without irradiation of the samples; (b) area of exposure and handling under refrigerated conditions with irradiation of the samples.***

### 1.2.2 Irradiation and control protocols for non-irradiated samples

The monitoring activity was organized in 8 study lines simulating possible real operational scenarios. Among these, some present more penalizing operating conditions such as the non-refrigerated environment and the absence of gloves during the handling operations of the substrates. In detail, the study lines are organized as follows:

- ✓ line n. 1 substrates open at 0 h time, exposed for a suitable time interval to environmental conditions with periodic manipulation;
- ✓ line n. 2 substrates open at 0 h time, exposed for a suitable time interval a environmental conditions, irradiated with periodic manipulation;

- ✓ line n. 3 substrates open at 0 h time, exposed for a suitable time interval to refrigerated conditions with periodic manipulation;
- ✓ line n.4 substrates open at time 0 h periodic manipulation;
- ✓ line n. 5 substrates open at 0 h time, exposed for a suitable period of time to environmental conditions with periodic handling without gloves;
- ✓ line n. 6 substrates open at 0 h time, exposed for a suitable interval of time to environmental conditions, irradiated with periodic handling without gloves;
- ✓ line n. 7 substrates open at 0 h time, exposed for a suitable period of time to refrigerated conditions with periodic handling without gloves;
- ✓ line n. 8 substrates open at 0 h time, exposed for a suitable period of time to refrigerated conditions, irradiated with periodic handling without gloves.

***glossary***

*substrates: Hamburger*
*environmental conditions: temperature and humidity present in the laboratory chilled conditions: refrigeration chamber temperature (fridge) 4 ° C*
*periodic handling (with gloves): simulation of preparations by handling gloves with the fieldsoni for 30-60 s*
*handling without gloves: simulation of preparations by handling gloves without gloves 30-60 s*
*irradiated substrates: substrates placed under light Biovitae® lamps (under the exposure conditions described and schematically reported below*

It was decided to start the study starting from line 5 for the most penalizing conditions set (absence of refrigeration and absence of gloves when handling the substrates), considering whether or not to proceed with the study of lines 1-4 in relation to the results obtained from the first observations to limit the consumption of hamburgers.

***Substrates line 5***

### 1.2.3 Sampling time, sample coding, sampling method

The monitoring timespan was set at 33 hours with a sampling frequency of 3 hours for lines 5 and 6 (activities at ambient conditions, therefore in more unfavorable conditions) and for longer intervals for lines 7 and 8 (activities in conditions refrigerated). Sampling plans are shown in Table 1.

***Table 1. Sampling plans.***

*lines 5 and 6*

| sample no | Time | Activities conducted |
|---|---|---|
| *First day* | | |
| 0 | 9:00 | Sensory and textural properties monitoring<br>Microbiological monitoring |
| 1 | 12:00 pm | Sensory and textural properties monitoring<br>Microbiological monitoring |
| 2 | 15:00 | Sensory and textural properties monitoring<br>Microbiological monitoring |
| 3 | 18:00 | Sensory and textural properties monitoring<br>Microbiological monitoring |
| *Second day* | | |
| 4 | 9:00 | Sensory and textural properties monitoring<br>Microbiological monitoring |
| 5 | 12:00 pm | Sensory and textural properties monitoring<br>Microbiological monitoring |
| 6 | 15:00 | Sensory and textural properties monitoring<br>Microbiological monitoring |
| 7 | 18:00 | Sensory and textural properties monitoring<br>Microbiological monitoring |

*lines 7 and 8*

| sample no | Time | Activities conducted |
|---|---|---|
| *First day* | | |
| 0 | 9:00 | Sensory and textural properties monitoring<br>Microbiological monitoring |
| 1 | 15:00 | Sensory and textural properties monitoring<br>Microbiological monitoring |
| 2 | 18:00 | Sensory and textural properties monitoring<br>Microbiological monitoring |
| *Second day* | | |
| 3 | 9:00 | Sensory and textural properties monitoring<br>Microbiological monitoring |
| 5 | 18:00 | Sensory and textural properties monitoring<br>Microbiological monitoring |

The hamburgers were irradiated with a known arrangement (Figures 4 and 5), providing for their labeling with codes referring to both the study lines and the exposure times (examples: L5 - T3hNI sample line 5 after 3 hours in non-irradiated conditions; L5 - T33hI sample of line 6 after 33 hours of irradiation). Some labeling acronyms shown in the figures have been made explicit in the captions.

At each sampling, the whole hamburger was divided into two parts to proceed with the sensorial and microbiological characterizations.

The sample at time zero (T0h) is the sample against which some comparison characterizations have been made (color, loss of free water). It was divided into the two sections for the indicated characterizations, immediately after removal from the refrigerator and the interruption of vacuum packaging.

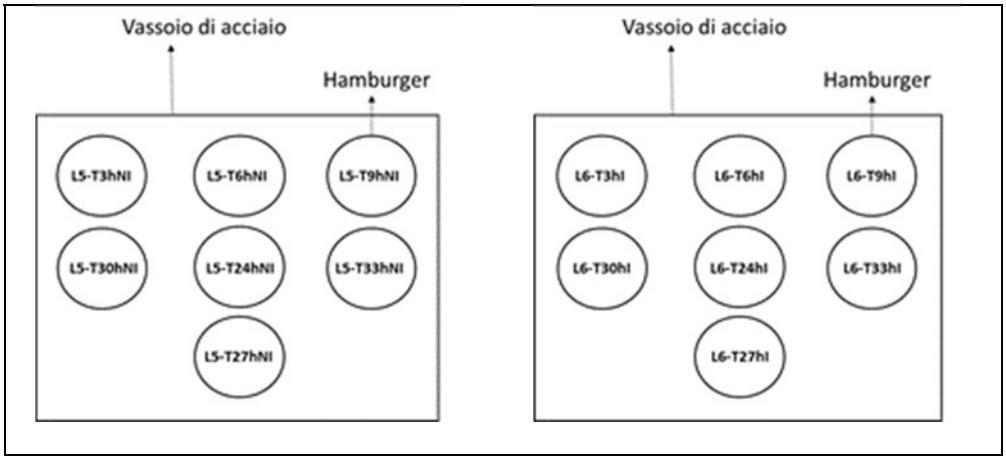



***Figure 4. Position of the samples on a steel tray for the area of exposure and handling at ambient conditions without radiation (left); position of the samples on a steel tray for the area of exposure and handling at ambient conditions with radiation.***

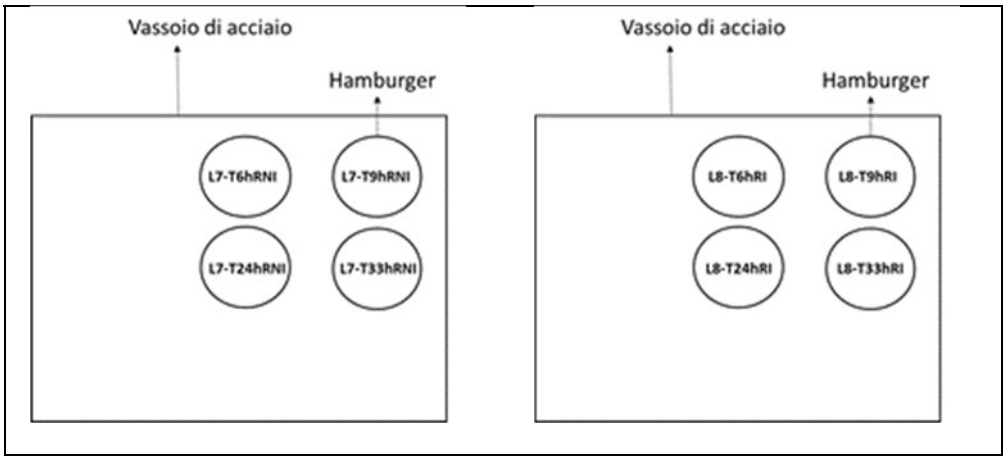



***Figure 5. Position of the samples on a steel tray for the area of exposure and handling under refrigerated conditions without radiation (left); position of the samples on a steel tray for the area of exposure and handling under refrigerated conditions with radiation.***

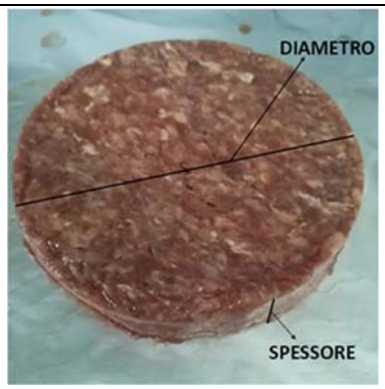
## 1.3 Sensory and textural properties monitoring results

### 1.3.1 Properties of the substrate in time zero

In zero time.

Table 2 shows some characteristics of a representative sample of time zero.

***Table 2. Average properties at time T0h of hamburgers.***




| | | |
|---|---|---|
| Diameter [cm] | | 10.9 ± 0.228 |
| Thickness [cm] | | 1:55 ± 0.118 |
| Weight [g] | | 151.5 ± 1:51 |
| Humidity [%] | | 46.5 ± 3:55 |
| Compactness | Max force [N] | 15.5 ± 0.588 |
| Color | L [-] | 47 ± 2:56 |
| | to [-] | 13.7 ± 1.66 |
| | b [-] | 16.7 ± 1:38 |

### 1.3.2 Shrinkage of substrates at the end of monitoring (lines 5-8)

As reasonably expected, for all the hamburgers examined during the application of the study protocols indicated with the denomination lines 5, 6 7 and 8, there were no significant shrinkage (textural contraction) measurements both for the diameter and for the thickness.

### 1.3.3 Loss of free water and compactness of hamburgers

Figures 6 and 7 show the values of the loss of free water and the maximum force for the evaluation of the compactness of the substrates in question exposed for a total of 33 hours to ambient conditions (L5, red color, Figure 6), to ambient conditions irradiated (L6, blue color, Figure 5), at refrigerated conditions (L7, pink color, Figure 7), at radiated refrigerated conditions (L7, green color, Figure 5), with periodic handling without gloves.

The loss of free water (loss of hamburger weight) was determined by the weighing method (see paragraph 2.1.2).

The evaluation of the compactness of the burgers was determined through the development of a protocol developed ad hoc (see paragraph 2.1.3). It consists, briefly, in registering the force necessary (indicated with Fmax) to impart a certain deformation value in compression (chosen equal to 50%) to the sample under examination: the more compact the sample the greater the force with the same induced deformation . The experimental values presented are averages of at least 3 measurements with standard deviations.

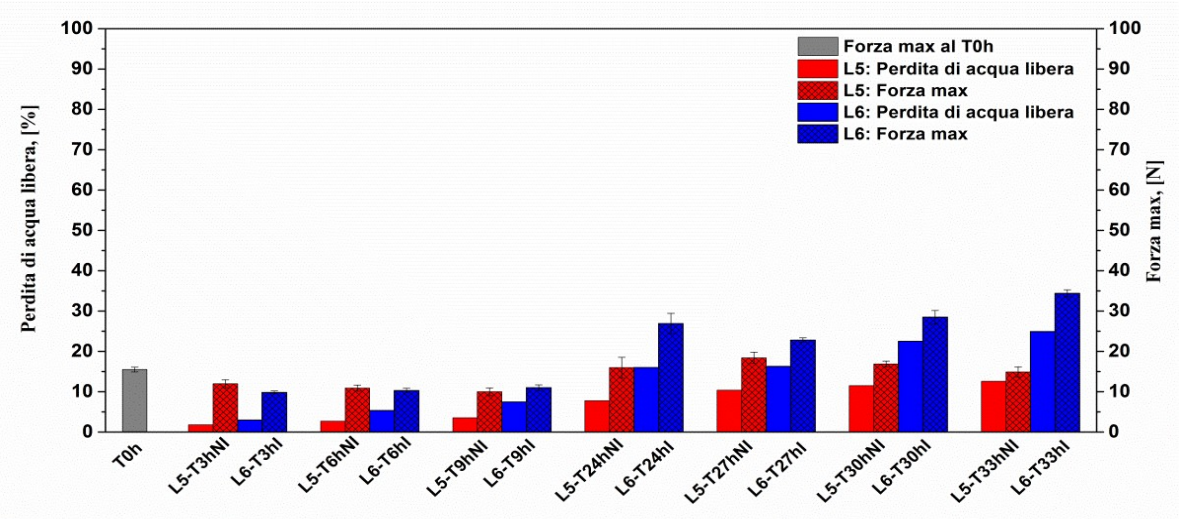



***Figure 6. Percentage of water lost and maximum force values as a function of monitoring time for substrates exposed for a total of 33 hours to ambient conditions with periodic manipulation without gloves (L5) and for substrates, exposed for a total of 33 hours at ambient conditions and irradiated with periodic handling without gloves (L6).***

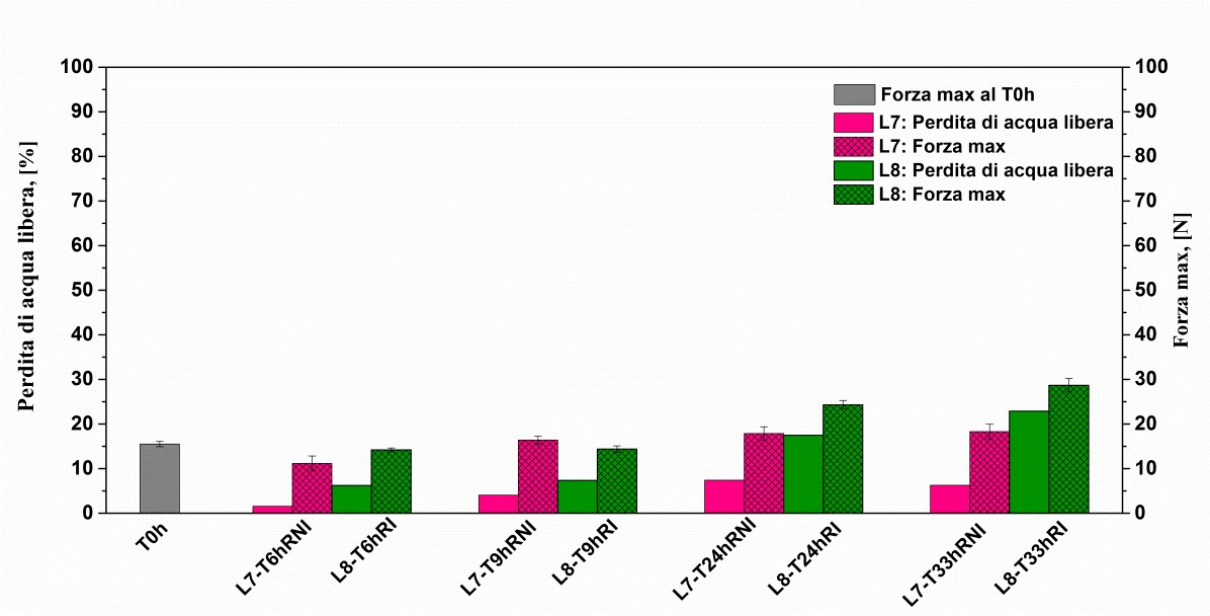



***Figure 7. Percentage of water lost and maximum force values as a function of monitoring time for substrates exposed for a total of 33 hours to ambient conditions with periodic manipulation without gloves (L7)) and for substrates, exposed for a total of 33 hours at ambient conditions and irradiated with periodic handling without gloves (L8).***

The results show that hamburgers exposed for a total of 33 hours to ambient conditions without irradiation (L5) do not have a significant variation ($p > 0.05$) of compactness (L5, Fmax, red color, Figure 6) and begin to show dehydration appreciable only after 24 hours of monitoring (L5, loss of free water, red color, Figure 6). The presence of the irradiation (L6), as reasonably expected, causes an increase over time in the loss of free water, however significant only after 24 hours, (L6, loss of free water, blue color, Figure 6) resulting, consistently, in a increased compactness (L6, Fmax, blue color, Figure).

A similar trend of results was obtained for the hamburgers exposed for a total of 33 hours to the chilled conditions (L7 and L8). In particular, the chilled conditions (T = 4 ° C) do not create a significant variation ($p > 0.05$) of the compactness of the hamburgers during the treatment hours (L7, Fmax, pink color, Figure 7) and the loss of free water after 9 hours reaches an almost constant value (L7, lost water, pink color, Figure 7). At radiated refrigerated conditions (L8) the hamburgers show greater compactness at 24 hours of monitoring (L8, Fmax, green color, Figure 7) because they lose a slightly greater water content (L8, loss of free water, green color,
Figure 7).

It is important to note that the values of Fmax at time T0h would seem not to follow the trends observed for subsequent samplings for all the monitored lines (L5, L6, L7 and L8). This result of greater firmness can be reasonably ascribed to the fact that the samples at the time zero are just extracted from the refrigerator (therefore colder) and not subjected to manipulation.

From the general comparison between the experimental data of the percentage of free water lost and of Fmax for the substrates of the lines L5, L6, L7 and L8 (see Figures 8 and 9, respectively) it is possible to highlight that the presence of the radiation (L6 and L8 ) determines a greater loss of water from the substrates, and therefore a greater compactness of the hamburgers compared to those that have not undergone radiation (L5 and L7), appreciable in any case starting from 24 hours of monitoring.

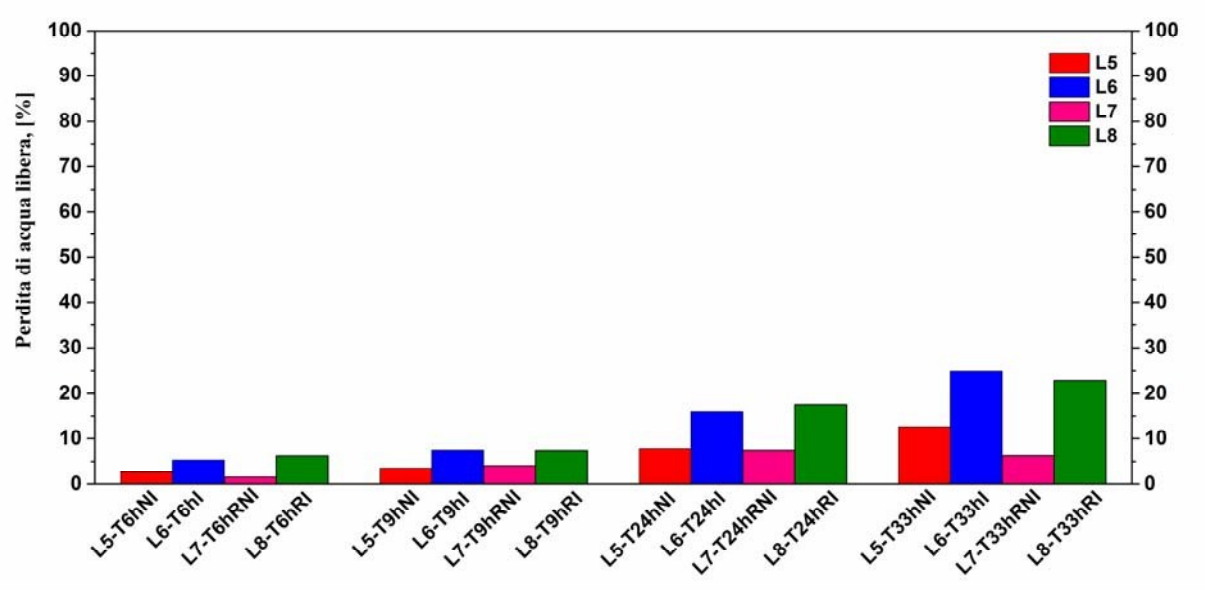



*Figure 8. Percentage of water lost as a function of monitoring time for substrates of lines L5, L6, L7 and L8.*

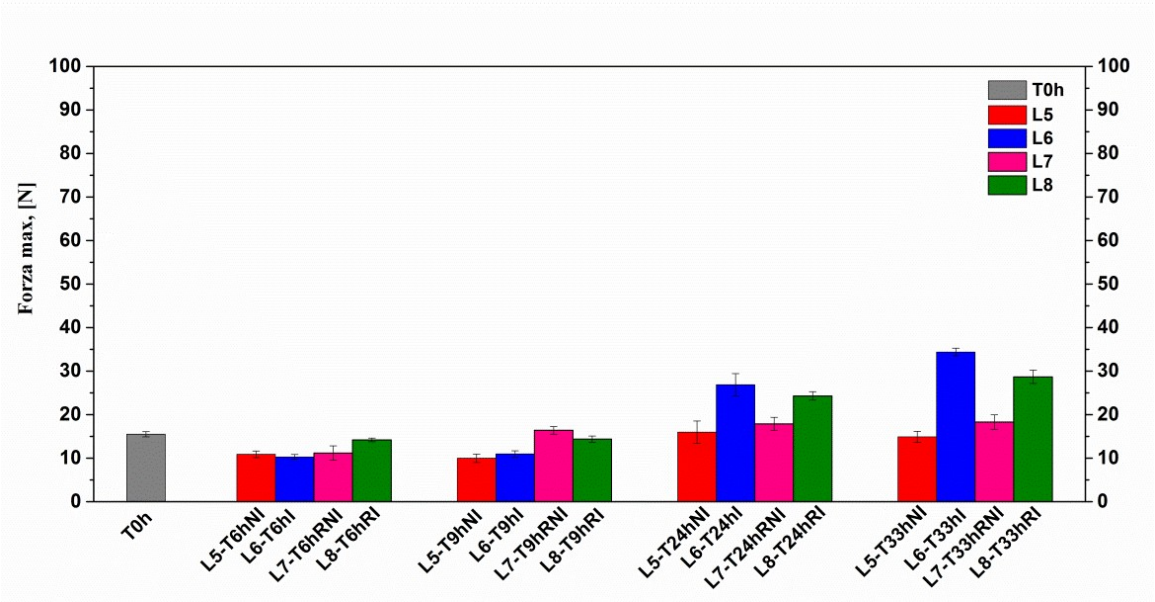



*Figure 9. Max force as a function of monitoring time for substrates of lines L5, L6, L7 and L8.*

### 1.3.4 Color variation

Figure 10 and Figure 11 show the color variation (ΔE) and brightness (L) values for the substrates exposed for a total of 33 hours to ambient conditions (Figure 10, L5, red color), to ambient conditions irradiated (Figure 10, L6, blue color), at refrigerated conditions (Figure 11, L7, pink color), at radiated refrigerated conditions (Figure 11, L8, green color). The variations in color and brightness were measured with the methods described in paragraph 2.1.4[2].

The results show that, for all monitoring times, hamburgers exposed to irradiated ambient conditions (L6, ΔE, red color, Figure 10) have a greater color variation than that obtained for

[2]In short: L indicates brightness (100 = white; 0 = black); the numerical value ΔE indicates color differences: <0.2 the difference is not perceptible; between 0.2 and 0.5 the difference is very small; between 0.5 and 1.5 the difference is small; from 2 to 3 there is a distinguishable color variation; from 3 to 6 the difference is quite distinguishable; between 6 and 12 means a strong color difference, typical of poor quality systems; > 12 means different colors.

hamburgers exposed to ambient conditions without irradiation ( L5, ΔE, red color, Figure 10). In particular, in the environmentally irradiated substrates, a difference in color (6 <ΔE <12) can be noticed already at 9 hours of monitoring. On the other hand, in non-irradiated ambient conditions, an appreciable color difference occurs only after 24 hours. These results are consistent with the values of the change in brightness (L) over time (Figure 10): the substrates processed under the conditions of the L6 line (L6, L, blue color,

In refrigerated conditions (4 ° C), in the presence and absence of radiation, the substrates show a fairly distinguishable color difference in the first 12 hours of monitoring (3 <ΔE <6), which becomes more pronounced at 24 hours (6 < ΔE <12). The greatest color variation was obtained at 33 hours of treatment for the irradiated refrigerated hamburger: the color of the substrate is different from that at time T0h (ΔE> 12), as can also be seen from the decrease in brightness (L in Figure 11) .

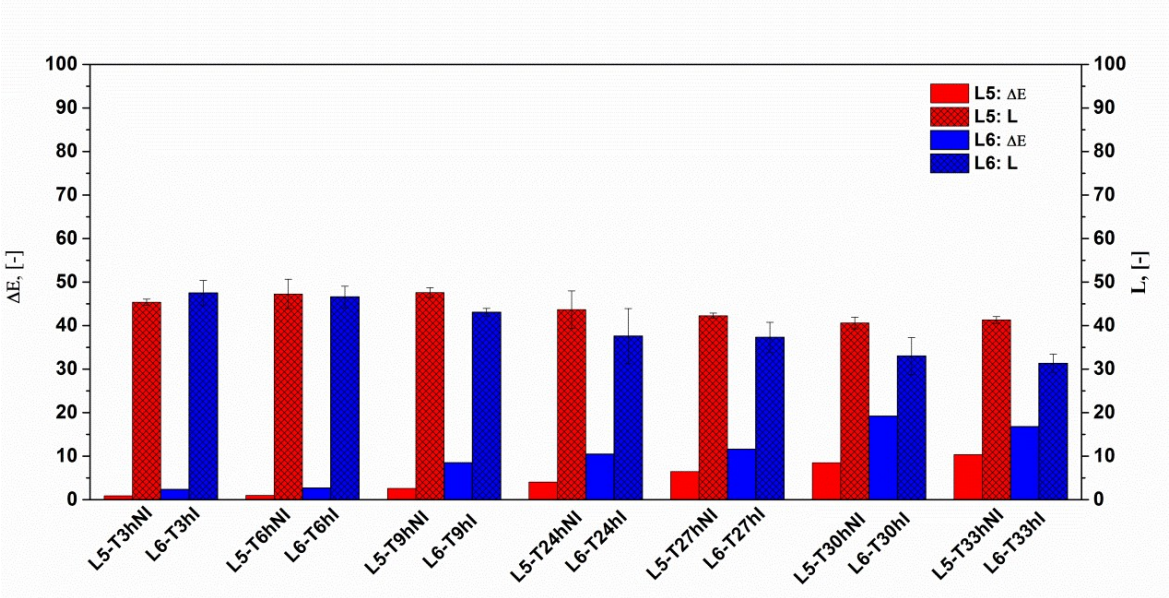



***Figure 10. Color variation (ΔE) and brightness (L) as a function of monitoring time for substrates exposed for a total of 33 hours to ambient conditions with periodic manipulation without gloves (L5) and for substrates, exposed for a total of 33 hours to environmental conditions, irradiated with periodic handling without gloves (L6).***

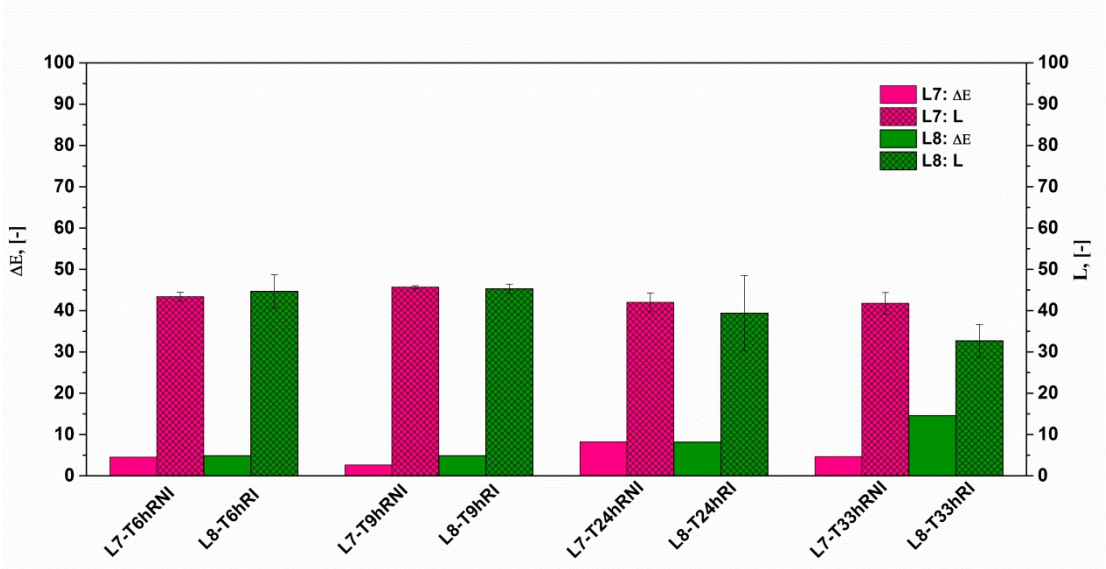



***Figure 11. Color variation (ΔE) and brightness (L) as a function of time for substrates exposed for a total of 33 hours under refrigerated conditions with periodic manipulation without gloves (L7) and for substrates, exposed for a total of 33 hours under refrigerated conditions, irradiated with periodic handling without gloves (L8).***

By comparing the experimental data of the color variation (ΔE) and brightness (L) for the substrates of the lines L5, L6, L7 and L8 (see Figure 12 and Figure 13, respectively) it is possible to highlight that all the burgers undergo a color change during the monitoring period (see Figure 12), regardless of the operating conditions used. The color variation is more marked for non-refrigerated irradiated substrates (L6).

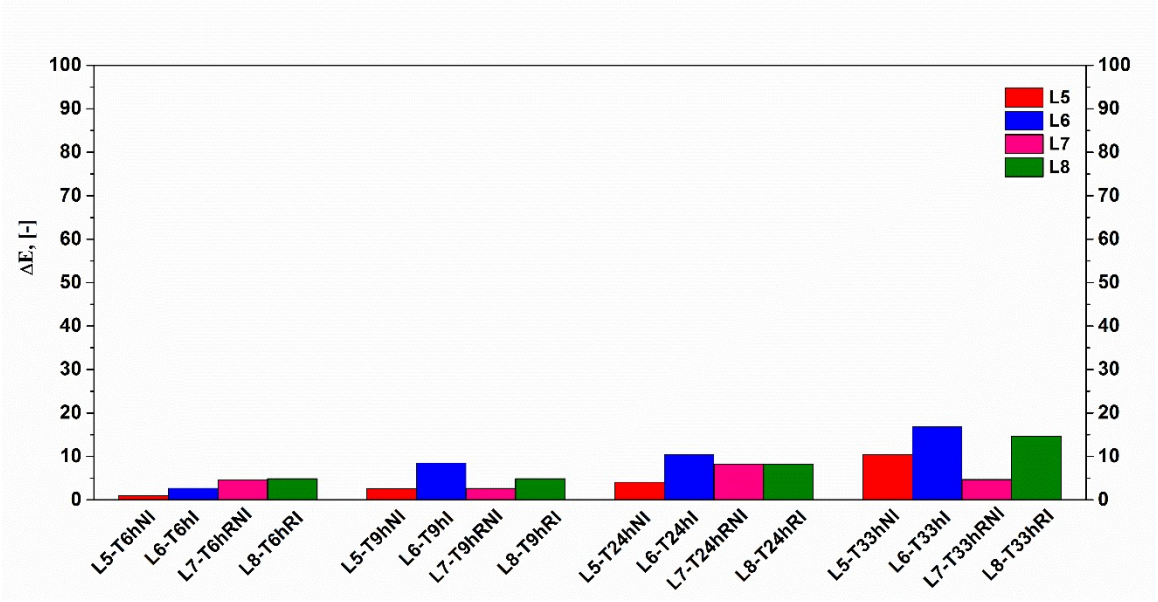



***Figure 12. Variation of brightness (ΔE) as a function of time for the substrates of the lines L5, L6, L7 and L8.***

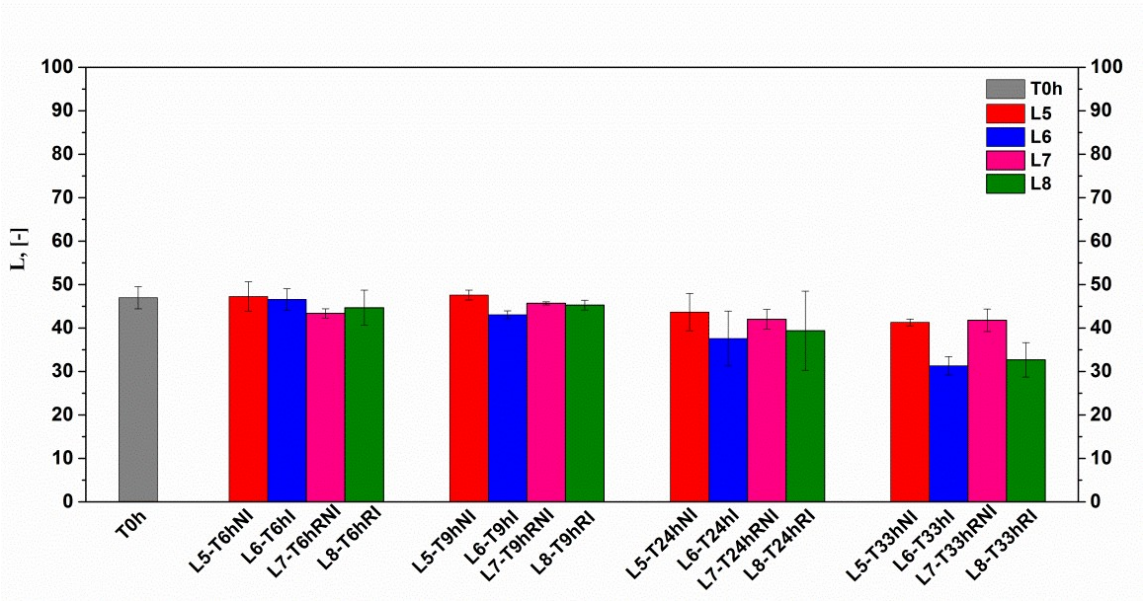



***Figure 13 Brightness (L) as a function of time for the substrates of the lines L5, L6, L7 and L8.***

### 1.2.5 Effect of the configuration of radiated loads on sensory properties and textures

The monitoring of the color and texture properties did not reveal any particular differences between the values obtained attributable to the different view factors in the study of the irradiated samples. However, it should be clearly pointed out that the different values of the free water losses and the different monitoring times do not allow to make clear observations on the role of the view factor (power density delivered to the load). In other words, the effect could be masked by the role played by the variation of other properties and for this reason the effect of the configuration of the irradiated loads on sensory properties and textures should be approached with a different methodological approach.

## 1.4 Microbiological monitoring results

The substrates under study (burgers) were subjected to microbiological monitoring in order to determine the ability of the LED light of the Biovitae® device to prolong its shelf life, i.e. the period of time in which the food stopped primary conservation (chain of the cold, vacuum), it can be kept in certain other storage conditions without suffering a decrease in the optimal levels of quality and safety. In particular, microbiological monitoring was aimed at evaluating the bacteriostatic capacity of the radiation protocols with the Biovitae® device.

At a regulatory level, the European Community has regulated microbiological controls for some types of food products through the EC Reg. 2073/2005 and subsequent amendments and additions (details in paragraph 2.2).

### 1.4.1 Total microbial load

The determination of the total mesophilic microbial load was carried out by applying the ISO 4833 protocol (details in paragraph 2.2)

As can be seen from the results shown in Figure 14, starting from time zero (T0h), the total mesophilic microbial load of the NRNI samples (red bars) gradually doubles in the first 9 hours of handling and storage at room temperature, and then decrease in the following hours. In particular, compared to T0h in the first 3 hours of handling at room temperature (T3h NRNI) the total microbial load increases by 72%; after 6 and 9 hours (T6h NRNI and T9h NRNI) it increases by about 120-125%. In the following hours (T24h, T30h and T33h NRNI) in all the analyzed samples the microbial load decreases with respect to T9h NRNI, but still remains slightly higher (about 30%) than the starting one of the T0h.

On the contrary, the samples handled at room temperature, but irradiated (NRI, blue bars) show, in the first 3 hours (T3h NRI) a slight increase in the total microbial load (about 30% compared to T0h) and then decrease in the following hours, reaching only 10% more than the starting microbial load (T0h).

The set of results obtained (UFC / g of total mesophilic microorganisms and UFC / g of Enterobacteriaceae) for the samples belonging to study lines 5 and 6, indicate that the action of the irradiation keeps the substrate microbiologically of satisfactory quality, while the non-irradiated substrate, from the first 3 hours (T3h NRNI), passes from satisfactory to acceptable quality
(see recommended guide values, Table 3, paragraph 2.2.2).

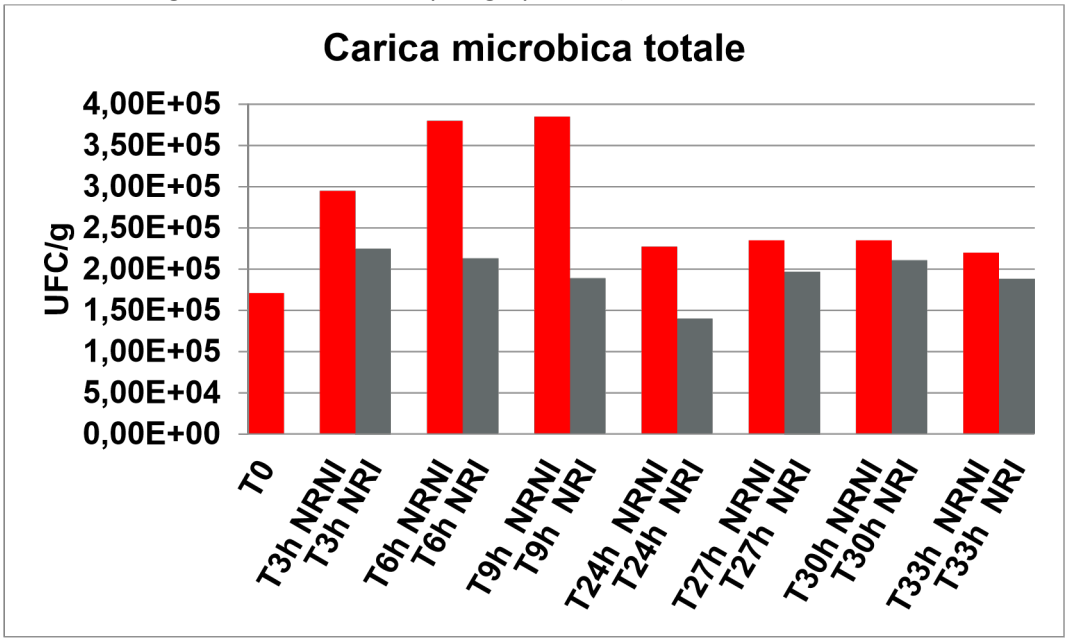



***Figure 14. Total microbial count in different samplings carried out over time for a total of 33 hours starting from substrates stored at room temperature with periodic manipulation without gloves and without irradiation (line 5, NRNI), or with irradiation (line6, NRI).***

Figure 15 shows the data obtained for the refrigerated substrates (study lines 7 and 8), for which the bacteriostatic action of refrigeration is evident. In fact, the UFC / g of total mesophilic microorganisms and the UFC / g of Enterobacteriaceae, for both samples, indicate that the substrates are microbiologically of satisfactory quality, up to 33 hours (T33h RNI and RI). In particular, in the non-irradiated RNI substrates (red bars), the total microbial load remains almost constant up to 9 hours of refrigeration and handling, with an increase of about 90 and 60% only after 24 and 33 hours respectively.

Naturally, the refrigerated and simultaneously irradiated NRI samples (blue bars) undergo the combined action of refrigeration and irradiation, as demonstrated by the fact that the total microbial load remains almost constant over time (with a slight increase of about 20% only after 24 hours of monitoring at 4 ° C).

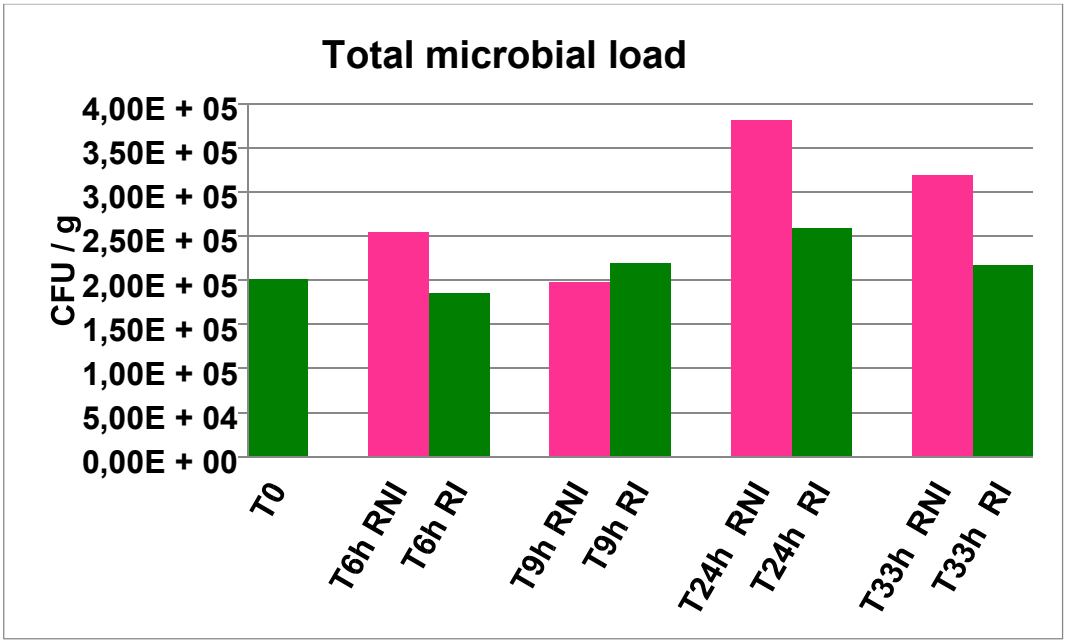



***Figure 15. Total microbial count in different samplings carried out over time for a total of 33 hours starting from refrigerated substrates with periodic manipulation without gloves and without irradiation (line 7, RNI), or with irradiation (line 8, RI).***

### 1.4.2 Enumeration of Enterobacteriaceae

Enterobacteriaceae enumeration was performed by applying the ISO 21528- protocol. 2: 2017 (details in paragraph 2.2)

The enumeration of Enterobacteriaceae also demonstrated the bacteriostatic capacity of LED light whose use has appreciably decreased microbial growth in the substrates analyzed. In fact, as shown in Figure 16 in the NRNI samples (red bars) the number of Enterobacteriaceae colonies increases by about 50% in the first 3 hours of handling and storage at room temperature (T3h NRNI) remaining high even in the following hours; on the contrary in the irradiated samples (blue bars), the Enterobacteriaceae charge decreases over time up to a value of about 20% at T24h NRI and about 50% after 33 hours (T33h NRI).

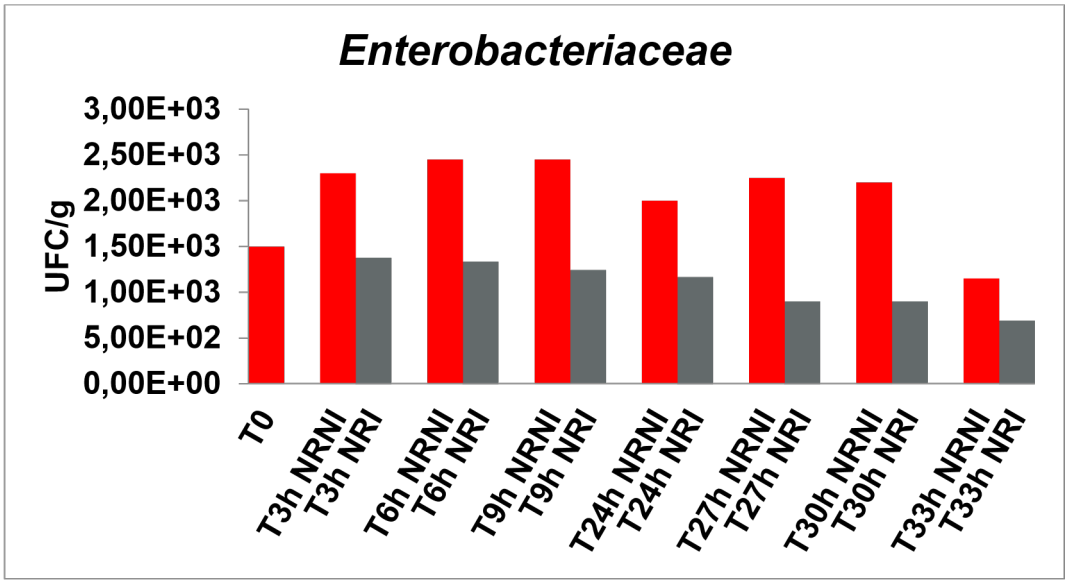



***Figure 16. Enterobacteriaceae count in different samplings carried out over time for a total of 33 hours starting from substrates stored at room temperature with periodic manipulation without gloves and without irradiation (line 5, NRNI), or with irradiation (line 6, NRI) .***

The Enterobacteriaceae count in the manipulated and non-irradiated RNI substrates (pink bars in Figure 17), showed an increase of about 48% in the first 6 hours compared to T0h. This increase in the number of Enterobacteriaceae colonies remains almost constant for up to 24 hours, then decreases slightly only after 33 hours (about 20% compared to T0h).

In manipulated and irradiated RI (green bars in Figure 17) refrigerated substrates, the number of Enterobacteriaceae colonies increases by only 15% in the first 6 hours, then decreases over time. In particular, after 33 hours the charge of Enterobacteriaceae decreases by about 42% compared to T0h.

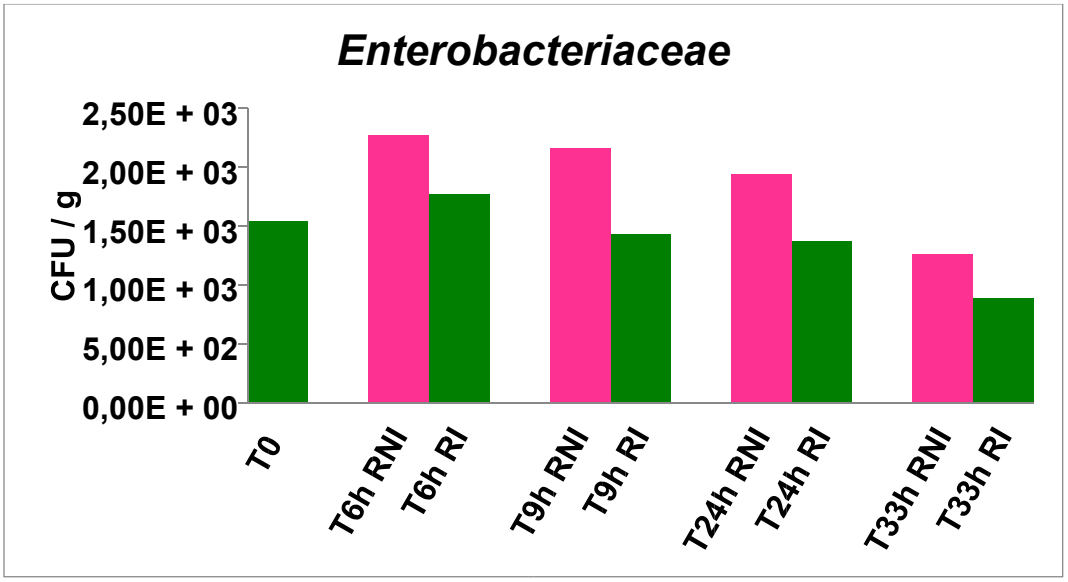



***Figure 17. Enterobacteriaceae count in different samplings carried out over time for a total of 33 hours starting from refrigerated substrates with periodic manipulation without gloves and without irradiation (line 7, RNI), or with irradiation (line 8, RI).***

The Enterobacteriaceae count in the manipulated and non-irradiated RNI substrates (pink bars in Figure 17), showed an increase of about 48% in the first 6 hours compared to T0h. This increase in the number of Enterobacteriaceae colonies remains almost constant for up to 24 hours, then decreases slightly only after 33 hours (about 20% compared to T0h).

In manipulated and irradiated RI (green bars in Figure 17) refrigerated substrates, the number of Enterobacteriaceae colonies increases by only 15% in the first 6 hours, then decreases over time. In particular, after 33 hours the charge of Enterobacteriaceae decreases by about 42% compared to T0h.

As for the total microbial load, it is important to note that, also for the growth of Enterobacteriaceae, the reduction of free water plays a not negligible role especially for the monitoring done at ambient conditions. However the bacteriostatic effect of the LED light is particularly well evident through the constantly lower counts compared to the non-irradiated samples.

### 1.4.3 Effect of the configuration of irradiated loads on microbiological monitoring.

Even for microbiological monitoring, there do not seem to be any particular differences in the detection of microbial charges attributable to the different view factors in the study of irradiated samples. As previously reported, however, the different factors such as loss of free water and the different monitoring times do not allow to make clear observations on the role of the view factor (power density delivered to the load).

## 1.4 General conclusions

From the point of view of texture and sensory aspects, the results of the various study lines show that the hamburgers exposed for a total of 33 hours to ambient conditions without radiation (L5) do not have a significant variation in compactness and begin to show appreciable dehydration only after 24 hours of monitoring. The presence of the radiation (L6), as reasonably expected, causes an increase in the loss of free water over time, consequently resulting in an increase in compactness. A similar trend of results was observed for hamburgers exposed for a total of 33 hours to chilled conditions (L7 and L8). In particular, the chilled conditions do not create a significant variation in the compactness of the hamburgers during the monitoring hours and the loss of free water after 9 hours reaches an almost constant value. At radiated refrigerated conditions (L8) the hamburgers show greater compactness after 24 hours of treatment because they lose a slightly greater water content. Finally, all monitored substrates undergo a slight to marked color change during the selected observation period (33 hours), regardless of the operating conditions used. The color variation is more marked for non-refrigerated irradiated substrates (L6). At radiated refrigerated conditions (L8) the hamburgers show greater compactness after 24 hours of treatment because they lose a slightly greater water content. Finally, all monitored substrates undergo a slight to marked color change during the selected observation period (33 hours), regardless of the operating conditions used. The color variation is more marked for non-refrigerated irradiated substrates (L6). At radiated refrigerated conditions (L8) the hamburgers show greater compactness after 24 hours of treatment because they lose a slightly greater water content. Finally, all monitored substrates undergo a slight to marked color change during the selected observation period (33 hours), regardless of the operating conditions used. The color variation is more marked for non-refrigerated irradiated substrates (L6).

**The set of results obtained from microbiological monitoring (UFC / g of total mesophilic microorganisms and UFC / g of Enterobacteriaceae) for the samples belonging to study lines 5 and 6 (non-refrigerated conditions), indicates that the irradiation action maintains the microbiologically satisfactory quality substrate, while the non-irradiated substrate, from the first 3 hours, passes from satisfactory to acceptable quality.**The data obtained for the refrigerated substrates (study lines 7 and 8) indicate that the bacteriostatic action of refrigeration has a key role allowing the substrates to maintain, from a microbiological point of view, a satisfactory quality for up to 33 hours. In particular, in the non-irradiated substrates the total microbial load remains almost constant up to 9 hours of refrigeration and handling, with an increase of about 90% and 60% only after 24 and 33 hours respectively. The refrigerated and simultaneously irradiated samples undergo the combined bacteriostatic action: the total microbial load remains almost constant over time showing a slight increase of about 20% only after 24 hours of monitoring. The Enterobacteriaceae count in the manipulated and non-irradiated refrigerated substrates showed an increase of about 48% in the first 6 hours compared to T0h. This increase in the number of colonies remains almost constant for up to 24 hours, then decreases slightly only after 33 hours. In manipulated and irradiated refrigerated substrates, the number of Enterobacteriaceae colonies increases by only 15% in the first 6 hours compared to T0h, and then decreases over time, demonstrating the bacteriostatic capacity of light **LED**.

For all types of monitoring performed in conditions of irradiation with LED light, it was not possible to identify the role, if any, of the different view factors. This is because the concomitant variation of other factors, such as loss of humidity and exposure times, does not allow making clear observations on the role of the visual factor (power density delivered to the load). In other words, the effect could be masked by the role played by the variation of other properties and therefore the relevance of the configuration of the irradiated loads is to be investigated with a dedicated methodological approach.

The results of all the monitoring carried out (study lines 5 - 8), especially those of a microbiological type, allow us to state that further investigations (study lines 1 - 4) conducted in a

similar way but with manipulation in the presence of gloves, not they would add more information, also involving only a consumption of resources.

## 2.1 Methods for monitoring textural and sensory properties

### 2.1.1 Shrinkage

The evaluation of shrinkage effects (contraction of the structure of the substrates under examination) was carried out by measuring the dimensions (diameter and thickness) of the hamburgers, with a calibrated ruler, at the initial time of the observation protocol and at the time of sampling.

### 2.1.2 Determination of initial and residual humidity and loss of free water

The moisture content of the hamburgers was determined using the Ohaus mod MB45 moisture analyzer. The humidity measurement of the sample is based on the thermogravimetric principle and was carried out according to ASTM D 2216–98: Standard Test Method for Laboratory Determination of Water (Moisture) Content of Soil and Rock by Mass. In short, after appropriate calibration , about 0.5 g of sample was loaded into an aluminum sample tray. When the measurement starts, a halogen lamp quickly heats the sample up to 200 ° C. During the test, the sample is constantly weighed by an internal balance, evaporative humidity losses are recorded and automatically reported as a percentage of residual humidity. At the end of the measurement, the percentage of residual moisture content (wet base) is obtained.

The determination of the loss of free water was carried out by means of a gravimetric method, that is, by weighing the samples at the initial time (mi) of the observation protocol and at the time of sampling (mt). In particular, the loss of free water was assessed with eq.
(1):

$$m\ m\ m$$

$$Perdita\ di\ acqua\ libera,\% \quad \_\_\_\_\_ \ 100 \qquad \text{eq. (1)}$$

### 2.1.3 Methods for monitoring textural properties

EN.CO's Texture Analyzer XT Plus was used to analyze the consistency of the hamburgers. The analyzer consists of a unit dedicated to measuring structural properties and a computer. The measurement unit consists of a work surface on which the samples to be analyzed are placed, a movable arm at the end of which it is possible to fix a measurement probe, chosen on the basis of the type of test to be performed, and of a support structure that allows the sliding of the mobile arm. By means of dedicated software, the remote management of the acquisition of the detected data and the transmission of the test setting as well as the calibration of the instrument takes place.

***Figure 18. Hamburger subjected to compression test using the Texture Analyzer XT Plus.***

In short, each sample underwent only one compression cycle using the flat-bottomed perspex cylindrical measuring probe with a diameter of 2 cm and a height of 4 cm. After calibration of the instrument, the sample was placed on the work surface and the compression test was started at a constant speed of 0.5 mm / s, inducing a sample deformation of 50% (see Figure 19). All acquisitions of the detected data were obtained using the dedicated Texture Exponent software, which records the resistance of the sample to deformation on a force-time diagram, also called the TPA curve (Texture Profile Analysis). The compactness of the burgers was determined from the force-time curve through the maximum force value ($F$) in the compression cycle (Figure 19).

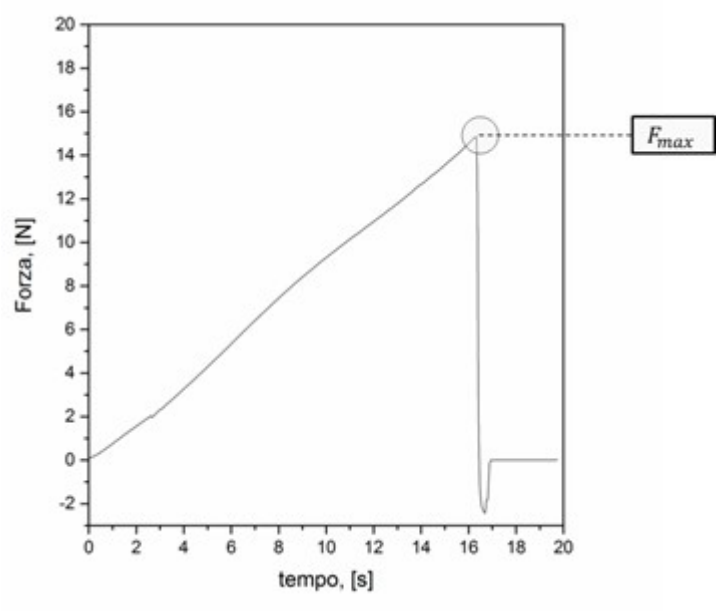



***Figure 19. Typical force-time curve in a hamburger compression cycle:*** $\boldsymbol{Fmax}$ is the force detected to induce the set deformation.

### 2.1.4 Color variation

A Konica Minolta SPECTROPHOTOMETER CM - 700d colorimeter was used to determine the color parameters of the hamburgers (see Figure 20).

***Figure 20. Konica Minolta SPECTROPHOTOMETER CM - 700d.***

The color of the samples was quantified according to the CIELAB standard illuminant (also known as Lab color space). In this color space, L indicates brightness (100 = white; 0 = black) while a and b the chromaticity coordinates (hue and saturation, respectively): + a is the direction of red, -a is the direction of green; + b is the direction of yellow and -b is the direction of blue.

In the Lab color space, it is possible to express the (visual) difference between two colors as a single numerical value ΔE according to the equation eq. (2)[3].

*[3]Papadakis, SE, Malek, SA, Tandem, RE, and Yam, KL "A versatile and inexpensive technique for measuring color of foods". 2000, Food Technol. 54 (12): 48.*

$$\Delta E \quad \sqrt{\quad \Delta L\, \Delta a\, \Delta b} \qquad \text{eq. (2)}$$

The following list of various ΔE values can serve as a guide for interpreting the extent of the color differences[4]:

- <0.2 the difference is not perceptible;
- between 0.2 and 0.5 the difference is very small;
- between 0.5 and 1.5 the difference is small;
- from 2 to 3 there is a distinguishable color variation;
- from 3 to 6 the difference is quite distinguishable;
- between 6 and 12 means a strong color difference, typical of poor quality systems;
- > 12 means different colors.

The instrument consists of a measuring head equipped with a display, functional keys and a power supply. The built-in light and dual beam feedback system ensure uniform illumination of the sample for all measurements. In short, after calibration of the instrument, the measuring head was placed on the sample surface in a vertical position and the light source was activated. In a few seconds the estimated values of the chromatic parameters were shown on the display and used to determine the color variation (ΔE) of the sample over time according to eq. (2), wherein Δ is the difference between the color of the substrate at T0h and the color of the same substrate at the time of the analysis.

### 2.1.4 Statistical analysis of data

The experimental data of the sensory and textural determinations were compared using the T-test. p stands for probability and its value measures how likely it is that any difference observed between the samples compared is due to chance. Since p is a probability, it can take any value between 0 and 1: a value of p approaching 0 testifies to a low probability that the observed difference can be ascribed to chance. In particular, p <0.05 means that the observed difference is not due to chance, i.e. there is a statistically significant difference between the two samples compared, on the contrary, p> 0.05 the two samples are similar.

*[4] Marco Riva, Insights: the color of food and its measurement, DISTAM, University of Milan, http://www.imimagesecomputer.it/allegati/Il%20colore%20degli%20alimenti.pdf*

## 2.2 Methods for microbiological monitoring

### 2.2.1 ISO protocols

The analyzes were performed according to ISO methods based on traditional microbiology approaches. In particular, for the preparation of each sample coming from the controlled substrate, 10 g were weighed aseptically and placed in a bag for homogenization. The sample was diluted 1:10 with peptone water preheated to 30 ± 1 ° C. Serial base 10 dilutions were performed within 30 to 45 minutes using peptone water preheated to 30 ± 1 ° C. The aliquots from the various serial dilutions were plated both on PLATE COUNT AGAR (PCA) plates and incubated for 72 hours at 30 ± 1 ° C for the determination of the total mesophilic bacterial load (ISO 4833), and on VRBG agar plates and incubated for 24 hours at 30 ± 1 ° C for the enumeration of Enterobacteriaceae (ISO 21528-2:

To calculate the number N of colony-forming units (CFU) present in one gram of product, we used the following formula:

$$N \quad \frac{C}{(n1 + 0{,}1 \cdot n2) \cdot d} \qquad \text{eq. (3)}$$

where is it:

*C*is the sum of the colonies in the plates considered n1 the number of plates considered, for the first dilution considered; n2 the number of plates considered, for the second dilution considered; d is the dilution factor that corresponds to the first dilution considered.

### 2.2.2 Notes on regulatory references for the evaluation of microbiological quality

At a regulatory level, the European Community has regulated microbiological controls for certain types of food products through the EC Reg. 2073/2005 and subsequent amendments and additions.

According to this Regulation, the results of microbiological analyzes can be interpreted on the basis of the number of colony-forming units (CFU / g), as is the case, for example, for the process hygiene indicator bacteria and some potentially pathogenic microorganisms; in this case it is a "three-class" system: satisfactory, acceptable or unsatisfactory (the latter, in some cases, following the risk assessment, may also be "potentially harmful"). Table 3 Quality categories

| ***Category*** | ***Meaning*** |
|---|---|
| Satisfactory | The result indicates an optimal microbiological quality for the type of product. |
| Acceptable | The result is the acceptability from the point of view of the microbiological profile, but the level of presence of some microorganisms could indicate areas of improvement in the supply of raw materials or in the hygiene of production processes. |
| Not satisfactory | The result indicates a high level of microbiological contamination in relation to the type of product, highlighting problems in the supply of raw materials or in the hygiene of the production processes. |
| Potentially harmful | The result highlights quantities of microorganisms that make the product unsuitable for human consumption or potentially harmful in the case of bacteria included in food safety criteria. Certain presence of supply or process problems production and shortcomings in the management of self-control. |

In the "three-class" sampling plan, 2 reference values are indicated:

- **m**: limit value of the number of microorganisms below which product conformity is determined;
- **M**: maximum value of the number of microorganisms tolerated in the product.

For the "minced meat" substrate, the values m and M are as follows (n indicates the number of sample units per aliquot; c indicates the number of sample units in which a value between m and M is allowed):

▪Aerobic mesophilic microorganisms ISO 4833: m = 500,000 CFU / g; M = 5.000.000 CFU / g (n = 5, c = 2)▪Enterobacteriaceae ISO 21528-2: m = 1,000 CFU / g; M = 10,000 CFU / g (n = 5, c = 2)

| | | Satisfactory | Acceptable | Not satisfactory |
|---|---|---|---|---|
| **Aerobic mesophilic microorganisms** | ISO 4833 | <5x105 | 5x105 <X> 5x106 | > 5x106 |
| ***Enterobacteriaceae*** | ISO 21528-2: 2017 | <103 | <103 <x> 104 | > 104 |