UNIVERSITÀ DEGLI STUDI DI FOGGIA

DIPARTIMENTO DI SCIENZE AGRARIE, DEGLI ALIMENTI E DELL'AMBIENTE

PhD Course in

"INNOVAZIONE E MANAGEMENT DI ALIMENTI AD ELEVATA VALENZA SALUTISTICA"

(*XXIX Cycle*)

COORDINATOR: PROF. FRANCESCO CONTÒ

New technologies for sanitization of

fresh dairy products

Tutor: **PhD student**

Prof. Del Nobile Matteo Alessandro   *Lacivita Valentina*

Co-tutor:

Dott.ssa Conte Amalia

Index

Index of figures

Index of tables

Abstract

L'utilizzo di tecnologie non termiche per la conservazione degli alimenti (campi elettrici pulsati, luce pulsata, luce UV, irradiazione, ultrasuoni) potrebbe rappresentare una strategia alternativa per aumentare la stabilità dei prodotti freschi. Differenti studi sono stati riportati sull'utilizzo di queste tecnologie su vari alimenti (frutta, verdura, carne cruda e pesce, succhi di frutta e latte), ma pochissime informazioni sul Fiordilatte sono disponibili. Sulla base di queste considerazioni, potrebbe essere interessante valutare l'efficacia di queste tecnologie non termiche sul Fiordilatte, al fine di prolungare la sua shelf-life.

In questa tesi di dottorato è stato studiato il potenziale utilizzo di tecnologie non termiche per la sanificazione di formaggi freschi. In particolare, sono stati testati sul Fiordilatte l'efficacia di diversi trattamenti: la luce UV-C, la luce pulsata (PL), gli impulsi di luce ad alta intensità (HILP), un sistema costituito da ultrasuoni combinati con il vapore (SonoSteam), i raggi X e il plasma freddo mediante l'utilizzo di un sistema Plasma jet.

Per ogni strategia di conservazione, sono stati effettuati test preliminari *in vitro* utilizzando *Pseudomonas fluorescens*, (o un mix costituito da *P. fluorescens* e *P. putida*) e Enterobatteri come inoculo sulla superficie del Fiordilatte. Poi, nella prova di shelf-life i campioni non trattati e trattati sono stati confezionati con liquido di governo, conservati a 9±1 °C e periodicamente analizzati dal punto di vista della qualità microbiologica e sensoriale. I trattamenti con luce UV-C sono stati eseguiti in una cella termostatata dotato di 4 lampade UV-C, che emettono luce nella lunghezza d'onda tra 180-200 nm. I trattamenti con luce pulsata (PL) e impulsi di luce ad alta intensità (HILP) sono stati eseguiti utilizzando rispettivamente una unità di decontaminazione mobile (CLARANOR) e Steri-Pulse XL 3000 (XENON). Questi dispositivi sono dotati di lampade allo xeno, che emettono luce ad alta intensità nella lunghezza d'onda tra 100-1100 nm. I trattamenti con ultrasuoni combinati con il vapore sono stati eseguiti con un impianto pilota dotato di 6

ugelli, in grado di produrre in maniera simultanea vapore (90-95 °C) e ultrasuoni (20-40 kHz). Il trattamento a raggi X è stato eseguito con il dispositivo RS-2400 (Rad Source, USA) utilizzando tre diverse dosi (0,5, 2, 3 kGy). Mentre, il trattamento con plasma jet è stato effettuata utilizzando un sistema pilota dove il getto di plasma è montato in una piccola camera che permette di lavorare a pressione atmosferica. L'Elio o una miscela di Elio + Ossigeno sono stati utilizzati come gas per la formazione del plasma.

Per ogni strategia di conservazione, la crescita degli Enterobatteri e delle *Pseudomonas* spp. è stata presa in considerazione, essendo questi gruppi microbici i responsabili principali del deterioramento dei prodotti lattiero caseari.

Dalle prove preliminari con i campioni inoculati, la luce UV-C è stata in grado di decontaminare la superficie del Fiordilatte. È stata ottenuta un'estensione della shelf-life del 80% selezionando adeguate modalità di trattamento come l'esposizione del Fiordilatte ai raggi UV-C a 6.0 kJ/m^2.

Il trattamento con la luce pulsata (PL) ha permesso una riduzione della crescita microbica, soprattutto immediatamente dopo il trattamento ed è stato osservato che la carica microbica diminuisce con l'aumentare della intensità della luce. Durante il test di shelf-life i risultati confermano che il trattamento PL esercita una attività germicida iniziale, ma non è in grado di inibire la crescita microbica durante la conservazione.

Mentre, per il trattamento con impulsi di luce ad alta intensità (HILP) è stato osservato una riduzioni significativa (oltre 1 ciclo log) su Fiordilatte inoculato, con solo pochi secondi di trattamento (da 1 a 8 s). Durante il periodo di stoccaggio refrigerato, nei campioni controllo si è osservato un aumento delle *Pseudomonas* spp., mentre i campioni trattati (B = 2 s e D = 4 s) non hanno mai raggiunto il limite di accettabilità microbiologica (dopo 12 giorni di stoccaggio).

Nello studio effettuato con ultrasuoni combinati con il vapore tutti i dati del test *in vitro* hanno evidenziato che questa tecnica può essere particolarmente efficace per ridurre la contaminazione iniziale del Fiordilatte. Durante il periodo di stoccaggio per le *Pseudomonas* spp., i campioni trattati (B = 1 s e E = 6 s) non hanno mai raggiunto la soglia di accettabilità microbiologica (dopo 12 giorni di stoccaggio).

Le *Pseudomonas* spp. e gli Enterobatteri, non sono stati rilevati nei campioni irradiati (trattamento a raggi X), indicando che questi gruppi microbici alteranti sono stati completamente inibiti dal trattamento a raggi X, mentre sono cresciuti nel controllo. La shelf-life per i campioni 2 e 3 kGy è stata circa di 44 e 43 giorni, rispettivamente. Mentre il controllo è rimasto accettabile per circa 10 giorni.

Al contrario, i tempi di trattamento lunghi, la modalità di applicazione del plasma e l'inefficacia sono stati i problemi connessi al potenziale utilizzo del plasma jet. Pertanto, le indagini con questa tecnologia sono state interrotte.

In generale, per i parametri sensoriali il punteggio della qualità globale ha indicato un accettabilità dei campioni di Fiordilatte trattati (UV-C, PL, X-ray) e questi sono stati percepiti come paragonabile ai campioni di Fiordilatte non trattati (controlli).

L'efficacia di queste tecnologie è molto interessante, in quanto riduzioni microbiche significative sono state registrate nella maggior parte dei campioni trattati. L'applicazione di queste nuove tecnologie a livello industriale ha ancora bisogno di altre indagini per meglio valutare gli effetti su alcuni parametri nutrizionali e costi per lo scale-up.

Abstract

The adoption of non-thermal technologies (Pulsed Electric Field, Pulsed Light, UV light, Irradiation, Ultrasound) for food preservation could represent an alternative strategy to increase fresh products stability. Studies on these technologies are reported for various food (fruit, vegetables, raw meat and fish, fruit juice and milk), but very few information on Fiordilatte cheese are available. On the basis of these considerations, it might be interesting to evaluate the effectiveness of these novel non-thermal technologies on Fiordilatte cheese, in order to prolong its shelf-life.

In this PhD thesis was investigated the potential use of new technologies for sanitization of fresh cheeses. In particular the effectiveness of UV-C light, pulsed light, the high intensity light pulses (HILP), combined steam and ultrasound, X-rays and plasma jet treatments were tested on Fiordilatte cheese.

For each preservation strategy, preliminary *in-vitro* tests were conducted using *Pseudomonas fluorescens*, (or a mix *P. fluorescens* and *P. putida*) and Enterobacteriaceae as inoculum on cheese surface. Then, in shelf-life test untreated and treated cheese samples were packaged with brine, stored at 9±1 °C and periodically analyzed for microbial and sensory quality. The UV-C light treatments were performed in a thermostated cell equipped with 4 UV-C lamps, which emit light in the wavelength between 180-200 nm. PL and HILP treatments were performed by using a mobile decontamination unit (Claranor) and the Steri-Pulse XL 3000, respectively. These devices are equipped with xenon lamps, that emit high intensity light in the wavelength between 100-1100 nm. The combined steam-ultrasound treatments were performed with a pilot system equipped with 6 nozzles, which are able to simultaneously produce steam (90-95 °C) and ultrasound (20-40 kHz). The X-rays treatment was performed by using the RS-2400 (Rad Source, USA) to three different doses (0.5, 2, 3 kGy). While, the plasma jet treatment was performed using a pilot

system where the plasma jet is mounted in a small chamber allowing the control of the atmospheric pressure. Helium or a mixture Helium + Oxygen as a plasma forming gas, were used.

For each preservation strategy, growth of Enterobacteriaceae and *Pseudomonas* spp. was taken into account, being these microbial groups the main responsible for dairy products deterioration.

From the preliminary tests with inoculated samples, UV-C light was demonstrated to decontaminate surface of Fiordilatte cheese. A 80% shelf-life extension was achieved by selecting adequate processing conditions of exposure of Fiordilatte cheese to UV-C light (6.0 kJ/m^2).

PL treatment may allow a reduction of microbial growth, especially immediately after the treatment and was observed that the microbial count decreased with the increase in light fluence. During the shelf-life test the results confirm that the PL treatment exerts an initial germicidal activity but it is not able to inhibit microbial growth during storage.

Whereas, for HILP significant reductions (more than 1 log cycles) were observed on inoculated Fiordilatte cheese, with only a few seconds of treatment (from 1 to 8 s). During storage period in the control *Pseudomonas* spp. increased, while the treated samples (B= 2 s and D= 4 s) did never reach the microbiological acceptability limit (after 12 storage days).

In the study performed with combined steam and ultrasound, all data of *in-vitro* tests highlighted that this technique could be particularly effective to reduce the initial cheese contamination. During storage period for *Pseudomonas* spp., the treated samples (B = 1 s and E = 6 s) never reached the microbiological acceptability threshold (after 12 storage days).

Pseudomonas spp. and Enterobacteriaceae were not detected in the irradiated samples (X-ray treatment), indicating that these spoilage microbial groups were completely inhibited by the irradiation process whereas they proliferated in the control. The shelf-life for the sample 2 and 3 kGy was circa 44 and 43 days, respectively. While the control remained acceptable for *circa* 10 days.

On the contrary, the long treatment times, the plasma application mode and the ineffectiveness were the problems related to the potential utilization of plasma jet treatment. Therefore, the investigations with this technology were aborted.

In general, the overall quality score for the sensory parameters has indicated an acceptability of the treated Fiordilatte cheeses (UV-C, PL, X-ray) and these samples were perceived as comparable to untreated cheeses (control).

The efficacy of these technologies is very interesting because a significant microbial reductions were recorded in most of treated samples. The application of these novel technologies at industrial level still needs other investigations for better evaluate the effects on some nutritional parameters and costs for scaling-up.

1. Introduction

The dairy industry has always been one of the most important components of the Italian food system. According to the definition dictated by EC Reg. 1234/2007 to art. 114/Annex XII *"dairy products are products derived exclusively from milk, on the understanding that can be added to substances necessary for their manufacture, as long as they are not used to replace in whole or in part any of the components of milk"*. The great tradition of Italian cheese is also demonstrated by the high consumption, which totaled around 20 kg/per capita per year, taking fourth place in the world, preceded by Greece (27.5 kg/capita), France (25.8 kg/capita) and Germany (21.8 kg/capita) (ISTAT data, 2010). Among dairy products, *pasta filata* cheese are the major contribution that Italy has given to dairy industry worldwide. The international success of *pasta filata* cheese as specialty cheeses produced a boom in the production and imitation; abroad, in Europe and in America, in fact, the consumption and production of *pasta filata* cheese, overcame tendency for the development of any other kind of cheese. Specifically, among these products, Fiordilatte cheese is very appreciated, being a fresh product with spun soft dough, high moisture content (50 and 60%), usually packaged in brine to preserve the soft-springy texture during storage at 4°C (Kindstedt, Carić, & Milanović, 2004). A peculiar characteristic of Fiordilatte cheese is the originality of the technology, which consists of dual processing, cheese making and curd stretching.

More in detail, the basic technology of Fiordilatte cheese is based on the following steps:

- acidification enough thrust of the milk in the boiler, by fermentation or chemical;
- draining and acidification of the curd to a pH of about 4.9-5.3;
- curd stretching that is mechanical traction of the curd soured heated to obtain a fibrous mass;
- cooling cheese dough a giving the desired shape.

Although Fiordilatte cheese receives heat treatment during curd stretching that substantially reduces the microbial load, post-processing contamination by microorganisms may occur, causing cheese spoilage and reduction of shelf-life, or resulting in a possible risk for consumers' health (Spano et al., 2003). Enterobacteriaceae, coliforms, *Pseudomonas* spp. and other psychrophilic bacteria can become the dominant microbial population in milk and fresh cheeses (Cantoni et al., 2003; De Jonghe et al., 2011; Franciosi et al., 2011; Martin et al., 2011; Morales et al., 2005). During storage, cheese spoilage can cause defects and alterations by hydrolysing different casein fractions (Baruzzi et al., 2012) and releasing lipases and proteases that consequently influence the textural properties, the appearance, the color (Cantoni et al., 2003), the odor and the taste (Oommen et al., 2002).

Increased consumer awareness and demands for fresh products have pushed the dairy industry to explore and develop alternative processing to heat techniques that not only ensure the safety of the product but also enhance shelf-life, retain nutrients and maintain freshness and wholesomeness of the processed product. Improving the process efficiencies and economics are other drivers to these innovations. For this reason, dairy industry must be able to ensure a certain stability of fresh products. Numerous studies have proposed different approaches to increase Fiordilatte shelf-life. For example, Conte et al., 2007 successfully investigated a release system based on lemon extract to prolong shelf-life of Fiordilatte cheese; Sinigaglia et al., 2008 demonstrated the antimicrobial effectiveness of lysozyme and Na_2–EDTA, dissolved in brine, in prolonging the Fiordilatte storability. In other study Gammariello et al., 2008 demonstrated that some essential oils, dissolved in brine, exerted an inhibitory effect on the microorganisms responsible for spoilage of Fiordilatte cheese. Other studies were conducted on the use of coatings and modified atmosphere packaging to control microbial proliferation and sensory changes (Mastromatteo et al., 2015b; Nardiello et al., 2014; Del Nobile et al., 2010; Gammariello et al., 2011). Conte et al., 2008 successfully investigated the combination of coating as a carrier of natural antimicrobials (lysozyme and EDTA) to MAP conditions in a sealed packaging system as a strategic solution to prolong the shelf-life of Fiordilatte cheese.

In the scientific literature, an integrated approach to prolong the shelf-life of Fiordilatte was reported and the new strategy was based on the combination of chitosan in the

manufacture. For example, Altieri, et al., (2005) successfully tested the use of chitosan as an antimicrobial agent in the process of Fiordilatte cheese; Del Nobile et al., 2009, investigated the combination of chitosan, coating and modified atmosphere packaging for prolonging Fiordilatte cheese shelf-life.

Currently, dairy sector is continually looking for new technologies that to respond to consumer requirements. An alternative strategy for fresh cheese preservation could be represented by the adoption of non-thermal technology. The development of non-thermal technologies have been introduced as a physical alternative method to slow or prevent the growth of microorganisms and preserve food quality. Researchers are studying non-thermal processing methods (methods that do not use heat) that are able to destroy pathogens and keep foods safe to eat, while retaining the sensory attributes and nutrient content similar to raw or fresh products. These alternative processing methods are at various stages of development, and have the potential to destroy pathogens and retain desired food quality.

The kinds of non-thermal processing methods that are currently being explored for a variety of ready-to-eat products to retain fresh attributes of food while ensuring safety are:

- High Pressure Processing (HPP)
- Gases (ozone, chlorine dioxide, cold plasma)
- Light (ultraviolet, pulsed light)
- Ionizing radiation (gamma irradiation, electron beam, X-ray)
- Ultrasound
- Pulsed electric field (PEF)

The use of UV-C light has recently aroused great interest because it can improve food safety and quality with minor effects on product nutritional and sensory characteristics (Soliva-Fortuny & Martín-Belloso, 2003). UV-C light treatments exploit the radiation with wavelength from 200 to 280nm. Due to the low penetration depth of UV-C radiation in a food matrix, this technology is particularly suitable for the inactivation of food surface microorganisms and enzymes (Choudhary & Bandla, 2012; Manzocco et al., 2011). At high levels, UV light causes damage to a microorganism's DNA. It is this characteristic of

UV light that is used to kill pathogens that are contained in food but does not impart any health concerns to the food. Studies on UV-C light treatments have been reported for minimally processed fruit and vegetables (Manzocco et al., 2015; Gómez et al., 2010), raw meat, fish, shell eggs, bakery products (Bintsis et al., 2000; Siddiqui et al., 2011) and liquids such as fruit juice, apple cider or milk (Matak et al., 2005; Franz et al. 2009; Bandla et al., 2012; Rossito et al., 2012; Cilliers et al., 2014).

High intensity light pulse (HILP) can be considered an alternative treatment for rapid and effective microbial inactivation in solid and liquid foods (Oms-Oliu et al 2010b). It is a non-thermal technology that uses short, intense pulses of white light in the wavelength range of 200–1100 nm, which includes ultraviolet, infrared and visible light. Treatment of foods with pulsed light (PL) has been approved by the FDA (21 CFR179.41). PL is the same as the light seen outside, but it is much more intense. When this light is flashed on a food, it kills microorganisms but has minimal impact on the food. Short flashes of this intense light are used to prevent the temperature of the food from increasing. The effects of HILP on microorganisms are mostly due to the photochemical action of the UV-C part of the light spectrum, the photothermal and photophysical effects for the high energy imparted. These mechanisms can cause an instantaneous heat generation inside the product, rupture of the cell wall and membrane, inactivation of bacteria due to UV absorption and DNA damage with eventual cell death (Gómez-López et al., 2007; Miller et al., 2012). Studies on HILP treatments have been reported for fruit and vegetable (Ramos-Villarroel et al., 2011, 2012 a,b; Oms-Oliu et al., 2010 a; Izquier, & Gómez-López, 2011), beverages such as fruit juice and milk (Palgan et al., 2011; Caminiti et al., 2011, 2012).

Ultrasound is considered a mild non-thermal technology. It is one of the new preservation techniques that could eliminate microbial activity. Its application in food processing is relatively recent. It appears to have a low lethality at low temperature but in combination with heat it can improve its microbial inactivation efficacy (Adekunte et al., 2010; Cameron et al., 2009; Gera & Doores, 2011). An example of a combined use of heat and ultrasound was proposed by SonoSteam (European patent EPO; 02 722 020.12113). The SonoSteam system uses the combination of steam (90-94 °C) and ultrasound (30-40 kHz), produced simultaneously by specially designed nozzles.

The inactivation effect of ultrasound was attributed to the generation of intracellular cavitation and these mechanical shocks can disrupt cellular structural and functional components up to the point of cell lysis (Chemat et al., 2011). The simultaneously application of steam and ultrasound improves cavitation efficacy, thus promoting rapid heat transfer into the surface of the product within a few seconds (Musavian et al., 2014; Hansen & Larsen, 2007; Boysen & Rosenquist, 2009). Studies on combined steam-ultrasound have been reported for decontaminate chicken carcasses (Hansen & Larsen 2007; Musavian et al., 2014), food boxes and other materials (Musavian et al., 2015).

Food irradiation has been identified as a safe technology on food process and used for disinfecting and preserving foods, most notably extending shelf-life (Akram et al, 2012; Tripathi et al, 2013, Komolprasert and Morehouse 2004). Gamma-ray, X-ray, and electron beams are called ionizing radiations, because they are capable of producing ions, electronically charged atoms or molecules. They have the same mechanisms in terms of their effects on foods and microorganisms. The principal target of ionizing radiation is water that produces free radicals, which react, destroy or deactivate bacterial components (Allende et al., 2006b; Rico et al., 2007; Soliva-Fortuny & Martin-Belloso, 2003). In the U.S.A., the Food and Drug Administration has permitted the irradiation of sprout seeds up to a maximum dose of 8 kGy to improve hygienic safety (CFR 2000). Moreover, with the use of irradiation, products could first be packaged and then treated without compromising the microbial quality of the product.

Cold Plasma is composed of gas molecules, which have been dissociated by an energy input. It is constituted by photons, electrons, positive and negative ions, atoms, free radicals and excited or non-excited molecules that, in combination, have the ability to inactivate spoilage microorganisms and pathogens from contaminated objects, including fresh and processed food surfaces (Fernández et al, 2012). It is considered to be the forth state of matter in the world (Tendero et al, 2006). This flexible sanitizing method uses electricity and a carrier gas, such as air, oxygen, nitrogen, or helium. The primary modes of action are due to UV light and reactive chemical products of the cold plasma ionization process (Niemira, 2012). The presence of oxygen in carrier gas leads to the formation of (ROS) reactive oxygen species (Schwabedissen et al. 2007; Klockow and Keener 2009; Oehmigen et al. 2010; Arjunan and Clyne 2011). The generation of these species depends

on the critical control parameters such as gas pressure and composition, temperature, moisture and plasma excitation properties (Ehlbeck et al. 2011; Misra et al. 2011). The mechanism of microbial inactivation have been well studied, the main modes of action at the bacterial cell wall and membrane disintegration causing surface lesions (Green et al. 2012). The limitations of plasma process for food sterilization are that, in treatment of bulky and irregularly shaped food, restricted volume and size of the food should be considered and also microbial inactivation occur on the surface of the food being treated since plasma reactive species are limited to penetrate into foods (Song et al, 2009).

Concerning studies on Fiordilatte cheese very few information are available. On the basis of these considerations, it might be interesting to evaluate the effectiveness of these novel non-thermal technologies on this traditional italian product, in order to prolong its shelf-life.

UV-C light, Pulsed Light, High intensity light pulse, combined Steam-Ultrasound and X-ray appear as emerging non-thermal processes with enormous potential for food decontamination. These technologies might provide safe products, minimizing the effects of heat on food quality attributes. In this context, the use of these treatments for sanitization of Fiordilatte cheese might be a potential approach to prolong the shelf-life of this product, without affecting the sensorial characteristics. Previous to the industrial implementation, however, the effects on Fiordilatte cheese have to be characterized. First, basic information concerning the decontamination effects of these treatments on spoilage microorganisms have to be evaluated. Secondly, these technologies could induce changes in relevant quality parameters and it is necessary their evaluation. Finally, the optimal processing parameters should to be determined.

1.1 Fiordilatte cheese

Fiordilatte cheese belongs to the category of stretched curd, or "pasta filata" cheeses. By "pasta filata" is intended a dairy technique characterized by a double process:

- the cheese-making in itself, that implies the various phases of coagulation, cutting, draining, shaping, salting and ripening (when necessary);
- the curd stretching, a treatment that renders the curd elastic.

Fiordilatte cheese is a soft, unripened pasta filata cheese manufactured from cow's milk using a variety of acidification methods, including direct acidification by addition of citric acid, or natural acidification by addition of thermophilic defined or undefined strain starters, including natural whey or milk cultures (De Angelis and Gobbetti, 2011). The Fiordilatte cheese is produced following a specific technological process. The specific technological phases have been also explained.

1.1.1 The raw material and its acidity

Cow milk is the raw material used to prepare Fiordilatte cheese. The acidity is a very important parameter for the production of Fiordilatte cheese. The quality of the cheese depends on its evolution. The acidity must be kept under control during all phases, from the milking to the stretching. If the milk has the right degree of acidity it coagulates well: the acidity and the temperature of the milk are the factors affecting the renneting in its coagulation effect. Should the curd not reach the right degree of acidity during the maturation phase, it may then not be possible to stretched it; the proper acidity allows the chemical-structural alterations that are necessary for the entire stretching process. The first thing to do is to determine the acidity of the milk that will be used to produce the Fiordilatte cheese. After having measured the acidity of the cow milk that usually it is between a score value equal to 6.6-6.8, it is possible to add:

1) natural whey culture;
2) industrial lactic acid bacteria;

3) citric acid, lactic acid or acetic acid

Natural whey culture

The natural whey culture that is used is the whey from the dairy process of the day before, left to acidify at 30-40 °C for 24 hours. To be more precise, the whey collected at the end of the ripening of the batch produced the preceding day is left to acidify. Before adding the natural whey culture it is necessary to determine the acidity of the milk and heat it up to 35°C. The acidity of natural whey culture is thus due to the action of these lactic acid bacteria that induce lactose to ferment (technically, one would say that lactose is the lactic acid bacteria's substrate). The result of this process is the lactic acid, thus acidity with all the related substances that will give the Fiordilatte cheese its characteristic aroma. The action of the lactic acid bacteria is called lactic acid fermentation. All is summed up in the following diagram:

Lactose ⟶ **Lactic acid + substances responsible for the aroma**

action of the lactic acid bacteria (lactic acid fermentation)
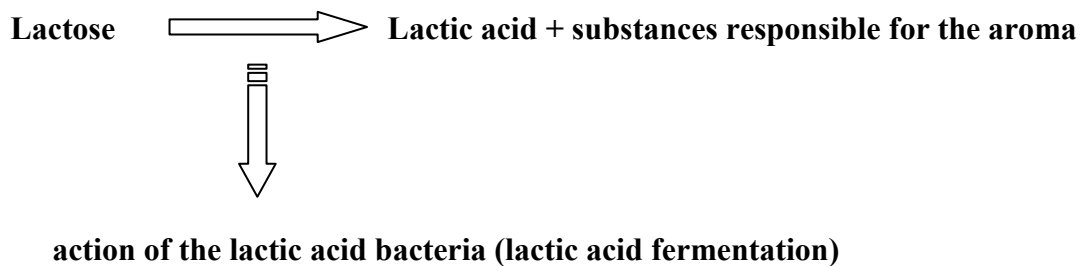
Industrial lactic acid bacteria

The natural acidification by addition of thermophilic strain starters (the industrial lactic acid bacteria) is based on the use of a variety of starter cultures dominated by *Streptococcus thermophilus* alone or a mixture of different lactic acid bacteria. It is sold as a powder (lyophilized) in metallic-plastic packets and dissolves very easily. To produce Fiordilatte cheese it is necessary to use thermophil enzymes that can withstand the high temperatures of dairy production; in this case must be present contain *Streptococcus thermophilus*, *Lactobacillus helveticus* and *Lactobacillus delbrueckii subsp. bulgaricus* (De Angelis et al., 2008; Coppola et al., 2006; de Candia et al., 2007).

Acids

In the direct acid addition process citric acid (or more rarely lactic acid) is added to pasteurized milk to obtain milk with an acidity of pH= 5.6, before rennet addition. In this case the curd is ready for stretching immediately follows the cutting of the curd the ripening phase is skipped; it is called immediate stretching (Faccia et al., 2009).

1.1.2 Coagulation

The milk is heated up to 36-38°C (this temperature promotes the coagulating effect of the enzymes that are in the rennet) and the rennet is added. The rennet is a mixture of various enzymes, each of which has a specific function on the milk's chemical elements (especially on its fats and proteins). The rennet can be of animal origin. It is extracted from the abomasum (stomach) of ruminants, specifically from calves, kids and lambs. Animal rennet can be industrial or farm-produced. The first one is usually liquid and mainly from calves. The quantity of rennet used each time will vary depending on the technology. The industrial rennet with a title of 1:10000 is used for making Fiordilatte cheese; the amount of rennet to be added varies between 15 to 20 ml/100 kilograms of milk and the coagulation time is about 15 minutes. When the curd reaches the proper consistency, usually the hardening takes 30-40 minutes and the cutting process starts. When speaking about coagulation time, we consider the whole process, including the setting and the hardening time. In the dairy it is possible to determine whether the hardening phase has finished by touching the surface of the mass with the back of the hand. If the hand remains dry, it means that the curd is dense and thus ready to be cut.

COAGULATION TIME

| Setting Time | Hardening |

renneting ≫≫≫≫≫ the first floccules appear ≫≫≫≫≫ compact and homogeneuous gel
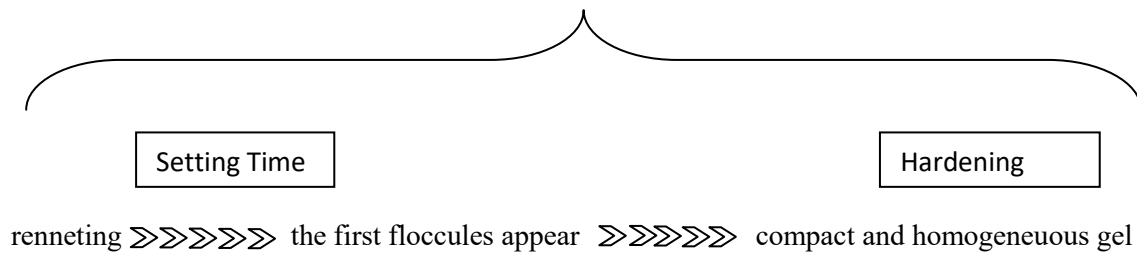
1.1.3 The cutting and curd maturation

The coagulation has transformed the milk (liquid state) into curd (gel state); more specifically, the curd contains whey - a liquid made up of water, lactose and proteins. The cutting process must be divided in two phases separated: in the first phase the curd is cut into big cubes during the following pause can be noticed the separation between whey and curd. During the second cutting (with a curd knife) the cubes are reduced into nut-size granules, soft and very moist because of the whey, that will give birth to a soft cheese. This phase is very important, because during the cutting some fat is always lost into the whey (part of the fat passes from the curd to the whey; there can be up to 1% of loss). A good cutting is thus fundamental to reduce these losses: it is best to always stir the curd gently. Having finished the cutting, another very important phase takes place: curd maturation. In the case in which the phase of acidification was performed with starter cultures, the maturation takes place under whey. Once the cutting is finished 60% of the whey is withdrawn. Lactic acid starts being produced during this rest (the pH value goes down and the curd becomes more acid); the acid captures the calcium ions (the mineral that allows casein to aggregate and thus form curd) and causes curd demineralization, which acquires flexibility. Practically, during the maturation takes place a reduction of the calcium in the curd with its increase in the whey. The optimal pH for the curd, at the end of the maturation stage, is of 4.9-5.2.

1.1.4 The stretching, shaping and packaging

The drained curd is ready for the stretching. The curd is cut into small pieces by a machine with rotary blades added hot water (80-85 °C) and stretching done with mechanic equipment. The stretching is the property of the curd that brought to high temperature becomes plastic and can be pulled in continuous filaments of length greater than one meter without losing excessive fat and moisture, passing from a granular discontinuous structure to a fibrous and continuous. The stretching is the phase of the processing that has the most influence on the consistency of the finished product and on the processing yield. The cheese is now ready for the shaping or "mozzatura" (the cutting of the curd into pieces of established size). The whole operation is totally mechanized (shaping machines) and when coming out of the dairy, the Fiordilatte cheese has a pre-established size. After the shaping, the produced Fiordilatte cheese is allowed to fall into containers or tanks filled with cold water to allow the cooling, as to insure that the cheese maintains its shape. After being cooled, the product is packed in plastic bags or trays and stored in governing liquid at 4 °C. This is usually mainly made up of tap water, brine and whey that preserve the soft-springy texture during cold storage for about10-12 days.

1.1.5 Fiordilatte cheese shelf life

Growth of a specific microorganism during storage depends on several factors, the most important being: the initial microbial loading at the start of storage; the physicochemical properties of the food, such as moisture content, pH, presence of preservatives; the processing method used in the production of the food; and the external environment of the food, such as the surrounding gas composition and storage temperature. A number of key intrinsic and extrinsic factors affecting the growth of spoilage organisms.

Psychrotrophic bacteria are the most commonly isolated organisms which caused the spoilage of the heat treated milk and dairy products as the result of post-pasteurization contamination of the products (Larsen and Jørgensen, 1997; Eneroth et al., 2000; Santana et al., 2004). The genus *Pseudomonas* are mentioned as the most often representatives of the Gram-negative population isolated from raw milk, with predominance of *P.*

fluorescens, that are the most commonly isolated spoilage organisms in raw and pasteurized milk (Samaržija et al., 2012).

In comparison to other psychrotrophic bacteria, *Pseudomonas* spp. are characterized by a short generation time (<4 h), which implies that contamination with just a single microbial cell can lead to their numbers greater than 10^6 cfu/mL in milk after eight days of storage at temperatures of 4 °C (Langeveld & Cuperus, 1976; Samaržija et al., 2012).

A proper combination of longer storage times and refrigeration temperatures creates an ideal growth environment particularly to psychrotrophic *Pseudomonas* spp., Enterobacteriaceae that can become the dominant non-lactic bacteria population in milk and in fresh cheeses such as Fiordilatte (Cantoni et al., 2003; De Jonghe et al., 2011; Franciosi et al., 2011; Martin et al., 2011; Morales et al., 2005). Their ubiquitous nature in the production environment and ability for rapid growth under low temperatures have made these groups of bacteria the leading direct and/or indirect cause of spoilage of milk and dairy products. Recently, the occurrence of very high loads of non-lactic acid bacteria populations, mainly composed of *Pseudomonas*, Enterobacteriaceae strains, was found to be responsible for different changes. For example casein hydrolysis, exfoliation of the outer surface of Fiordilatte, anomalous discoloration and in general sensory changes, such as generation of off-odours and flavours and changes in texture, frequently from the action of enzymes produced by microorganisms (Baruzzi et al., 2012; Martin et al., 2011; Cantoni et al., 2003; Caputo et al., 2015).

The activity of the proteinases of *Pseudomonas* spp., and especially *P. fluorescens* leads to the occurrence of sliminess and pigmentation in fresh cheeses. For example, it is confirmed that the slimy texture of fresh cheeses (with 55-80 % moisture content) is a direct result of the contamination of cheese with Gram-negative psychrotrophic bacteria, usually the species *P. flourescens, P. fragi* and *P. putida* (Brocklehurst and Lund, 1985; Fox et al., 2000; Samaržija et al., 2012). Indirectly, the poor texture of fresh cheeses is the result of the activity of the thermostable proteolytic enzymes of those bacteria.

Furthermore, official analyses linked the anomalous discoloration to contamination of Fiordilatte cheese with strains of *Pseudomonas fluorescens*, which derived mainly from water used during the processing. However, the high levels of *P. fluorescens* and occurrences of anomalous coloration are not consistent, because only some strains are able

to produce the pigment (Carrascosa et al., 2015). In fact, several cases of anomalous discoloration were reported in Fiordilatte cheese and referred to the contamination by *Pseudomonas putida* (reddish discoloration; Soncini et al., 1998), *Pseudomonas fluorescens* (greenish and fluorescent discoloration; Franzetti and Scarpellini, 2007), *Pseudomonas fluorescens* biovar IV and *Pseudomonas libanensis* (bluish discoloration; Cantoni et al., 2003), *Pseudomonas gessardii* (yellow-purple spots; Cantoni et al., 2006).

Contamination by *Pseudomonas* spp. and Enterobacteriaceae, as the result of post-pasteurization contamination , play an important role in milk and dairy products. Because these spoilage bacteria produce many lipolytic and proteolytic enzymes, which reduce both the quality and shelf-life of Fiordilatte cheese (Santana et al., 2004; Samaržija et al., 2012).

The *shelf-life* is the period of time during which the product maintains its quality characteristics, under normal conditions of storage and use. Many factors can influence shelf-life, and can be categorized into intrinsic and extrinsic factors (IFST, 1993). Intrinsic factors are the properties of the final product. They include the following:

- Water activity (a_w) (available water).
- pH value and total acidity;
- Available oxygen;
- Nutrients;
- Natural microflora and surviving microbiological counts;
- Natural biochemistry of the product formulation (enzymes, chemical reactants).
- Use of preservatives in product formulation (e.g. salt).

Intrinsic factors are influenced by such variables as raw material type, quality and product formulation and structure. Extrinsic factors are those factors the final product encounters as it moves through the food chain. They include the following:
- Temperature control during storage and distribution;
- Environmental microbial counts during processing, storage and distribution;
- Composition of atmosphere within packaging;
- Consumer handling.

In this contest Fiordilatte cheese is a very perishable products, it has high moisture content (50-60%) and was generally packaged in trays or pouches with brine and stored at 4 °C. Usually its shelf-life is around 1-2 weeks, but one of the most important factors which limits the shelf-life of Fiordilatte is the use of water as covering liquid. Under these storage conditions the quality rapidly deteriorates, due to microbiological and biochemical modifications. An important role is also played by diffusion phenomenon which give rise to strong matter exchange between cheese and liquid phase (Faccia et al., 2011).

Numerous studies have proposed different approaches to increase Fiordilatte shelf-life. Some studies have been conducted on the use of natural antimicrobial compounds. Conte et al., 2007 successfully investigated a release system based on lemon extract to prolong shelf-life of Fiordilatte cheese; Sinigaglia et al., 2008 demonstrated the antimicrobial effectiveness of lysozyme and Na_2–EDTA, dissolved in brine, in prolonging the Fiordilatte storability. In other study Gammariello et al., 2008 demonstrated that some essential oils, dissolved in brine, exerted an inhibitory effect on the microorganisms responsible for spoilage of Fiordilatte cheese. Other studies were conducted on the use of coatings and modified atmosphere packaging to control microbial proliferation and sensory changes (Mastromatteo et al., 2015b; Nardiello et al., 2014; Del Nobile et al., 2010; Gammariello et al., 2011). Conte, et al., (2008) successfully investigated the combination of coating as a carrier of natural antimicrobials (lysozyme and EDTA) to MAP conditions in a sealed packaging system as a strategic solution to prolong the shelf-life of Fiordilatte cheese.

Furthemore, in the scientific literature, an integrated approach to prolong the shelf life of Fiordilatte was reported and the new strategy was based on the combination of chitosan in the manufacture. For example, Altieri, et al., (2005) successfully tested the use of chitosan as an antimicrobial agent in the process of Fiordilatte cheese; Del Nobile et al., 2009, investigated the combination of chitosan, coating and modified atmosphere packaging for prolonging Fiordilatte cheese shelf-life. Afterwards, nano-systems containing silver nanoparticles (Ag-NP) have received great attention. Regarding to the application of Ag-NP to Fiordilatte cheese, few papers are reported in the scientific literature. Incoronato, et al., (2011) demonstrated a significant shelf-life prolongation of Fiordilatte cheese by means of Ag-NP incorporated in an agar hydro-gel. Also, Gammariello, et al., (2011)

indicated that a silver-based nanocomposite coating and MAP enhanced Fiordilatte cheese shelf-life packaged without the covering liquid.

An alternative strategy to preserve Fiordilatte cheese could represent by the adoption of non-thermal technology.

1.2 Non-thermal technologies

The ever increasing consumer demand for safe, fresh, convenient and high quality food has led to an increasing need to control the microbial quality of products. In this context, also dairy industry must be able to propose preservation techniques for fresh products that assure safety and quality to consumers. Therefore, the adoption of non-thermal technologies could be an interesting alternative for food preservation.

Alterative technologies for inactivation microorganisms without relying on heat are not new concepts, but their development for use as food preservation treatments has received considerable attention only recently, in response to need microbial food safety and quality, without compromising the nutritional and sensory characteristics of foods (Ross et al., 2003; Sale and Hamilton 1968; Sale et al., 1970). This novel non-thermal technologies are able to inactivate microorganisms at ambient or near ambient temperatures, without affecting the sensory attributes and nutrient content (Barbosa-Canovas et al., 1999).

There are several non-thermal techniques that have shown significant contributions in the food industry and the foods treated with non-thermal process are safer to eat then untreated products. These novel non-thermal processing include High Pressure Processing (HPP), different form of Ionizing radiation (Gamma radiation, Electron beam, X-ray), different form of light (Ultraviolet, Pulsed Light), Ultrasound (US) and different form of gases (Ozone, supercritical Carbon Dioxide, Cold Plasma). Studies on non-thermal technologies are reported for minimally processed fruit and vegetables (Manzocco et al., 2015; Gómez et al., 2011, Manzocco et al., 2011; Fernández et al., 2013; Wang et al., 2012; Tappi et al., 2014) raw meat and fish (Bolumar et al., 2011; Jofré et al., 2008; McDonnell et al., 2014), egg (Manzzoco et al., 2013) and liquids such as fruit juice (Thairi et al., 2006; Polydera et al., 2005, Franz et al., 2009) apple cider or milk (Cilliers et al., 2014; Innocente et al., 2014; Trujillo et al., 2002; Evrendilek et al., 2004).

1.2.1 Ultraviolet

1.2.1.1 Definition and general concept

Ultraviolet light can be described as a spectrum of light, remaining below the range visible to the human eye (Sosnin et al., 2006; Shama, 2007), as is presented in Figure 1. UV rays can be classified as Vacuum UV, UV-A, UV-B and UV-C based on the emission wavelength. Vacuum UV is in the range 100-200 nm, UV-A is defined as wavelengths between 315 and 400 nm, UV-B lies in the range 280-315nm and UV-C remains from 200 to 280nm.

ELECTROMAGNETIC SPECTRUM
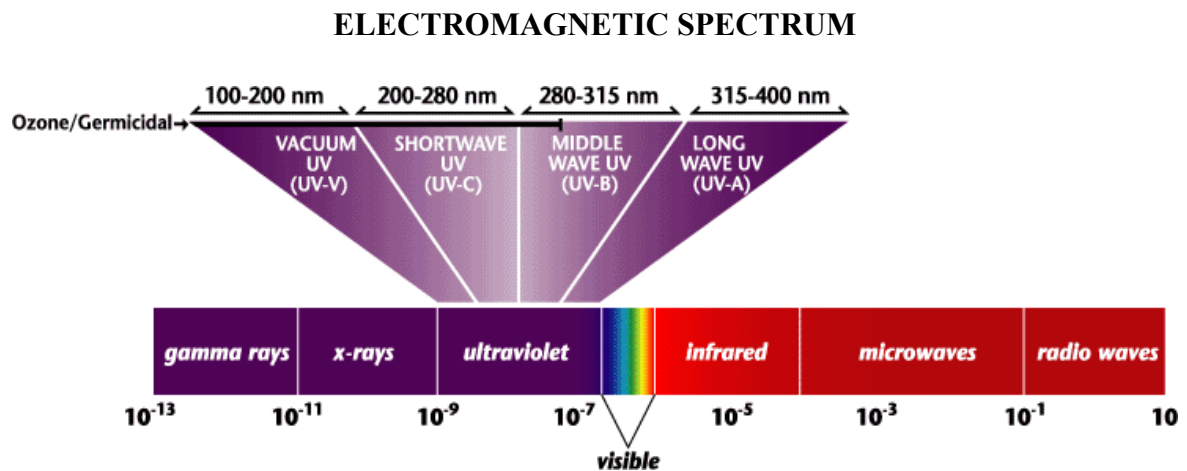
Figure 1 *Electromagnetic spectrum.*

The part of an electromagnetic spectrum, which is responsible for germicidal effect to microbial structure is located between 200 and 300 nm, is called UV-C (Shama, 2007). The UV-C spectrum (200-280 nm) is the most lethal range of wavelengths for microorganisms. The germicidal effect of ultraviolet radiation was first detected in 1878, but the first processing units were built only in 1955 in Switzerland and Austria.

Ultraviolet radiation, for the first time, was applied to the treatment of water and after for juices treatment. UV-C irradiation is widely used as an alternative to chemical sterilization

and microbial reduction in food products and has been approved for use as a disinfectant for surface treatment of food (US-FDA, 2002).

The technology of UV irradiation is applied, since 1930 in United States, on air and surfaces, in sterile environments like hospitals. Then it was adapted for packaging sterilization, such as bottle caps from high density polyethylene and paperboard for liquid products, yogurt containers, plastic cups and aluminum lids, besides the surface of fruits and vegetables to increase tissue resistance to spoilage microorganisms (Bintsis et al., 2000).

UV treatment is a physical method in which the energy is introduced either onto the food surface or into the liquid medium. The treatment system changes on the base of different applications. For liquid foods the treatment system usually comprises a reaction chamber for UV light treatment in the form of concentric tubing or other designed tubes, a UV-C lamp, containers for the liquids, plastic tubing, a refrigeration system and pumps. An UV lamp surrounded by a quartz jacket is placed inside the concentric tube system. The liquid flows through the annular part of the tube to achieve the required germicidal effect. Instead for solid foods the UV treatment system usually comprises a reaction chamber for UV light treatment, a UV-C lamp housing mounted on each side and a refrigeration system. Mercury lamps can be used as UV-C sources because of their electromagnetic emission at about 254 nm (Franz et al., 2009). Lamps can be constructed with glass or quartz, which allows the transmission of UV-C.

The efficiency of the UV process will depend on several factors like fluence, intensity, absorption coefficient, flow rate, turbidity and sample depth. The fluence is the amount of UV light exuded from the germicidal bulb and will depend on light intensity and exposure time. The intensity is an inherent characteristic of the lamp, but the intensity arising to the sample is conditioned to the bulb strength and geometry of the reactor (distance between UV source and sample). The absorption coefficient delineates how much light is lost as it passes through a medium, and is defined by the Lambert-Beer law (Equation 1):

$$A = \varepsilon \cdot d \cdot c$$

<div align="right">(Eq. 1)</div>

Where **A** is the absorbance, **ε** is the molar extinction coefficient, **d** is pathlenght in cm, and **c** is the molar concentration.

The UV irradiation intensity is expressed as irradiance or intensity flux (W/m). The dose (Equation 2) is a function of the intensity and the exposure time, expressed as radiant exposure or dose (J/cm) (Bintsis et al., 2000; Matak et al., 2005; Keyser et al., 2008).

$$D = I \cdot t$$

<div align="right">(Eq. 2)</div>

Where **D** is the treatment dose (J/cm), **I** is the intensity or dosage rate (W/m) and **t** is the contact time for UV treatment (s).

1.2.1.2 UV treatment on microorganisms: mechanisms of action.

Microbial inactivation is caused by a significant effects involving DNA mutation (Sastry et al., 2000; Guerrero-Beltran and Barbosa-Canovas, 2004). Photochemical damage to nucleic acids in cellular structure is the main result of the absorption of germicidal UV-C light. The germicidal property of UV light is mainly due to alterations to the microbial DNA caused by cross-linking of the pyrimidine bases with the formation of cyclobutyl pyrimidine dimers (Figure 2). Dimerization of adjacent pyrimidine molecules is the most common photochemical damage, the dimers block DNA transcription and replication by compromising cellular functions causing other types of damage, such as cross-linking of nucleic acids and proteins, results on cell death (Ball 2007; Tornaletti 2005; Guerrero-Beltran and Barbosa-Canovas, 2004; Guneser and Karagul Yuceer, 2012). The extent of cross-linking is proportional to the treatment dose of UV light.
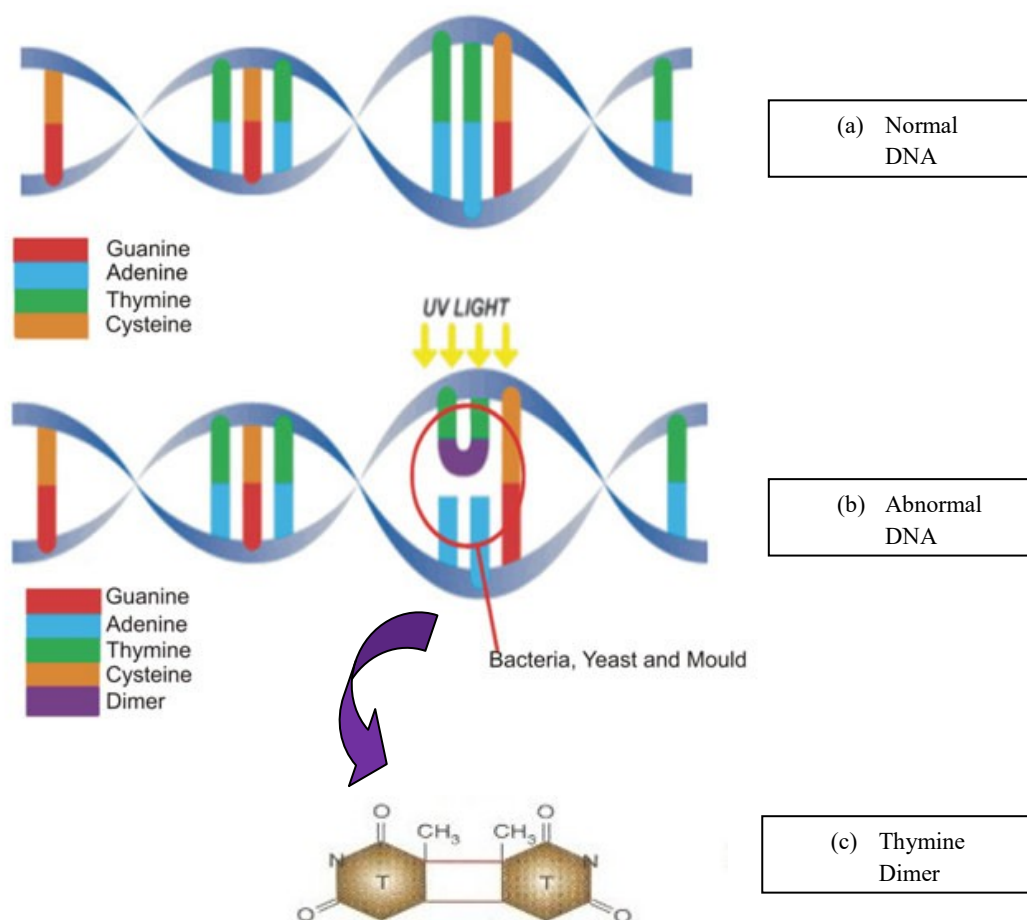
Figure 2 Microbial DNA damage after UV exposure.

The amount of cell damage depends on the dose of UV energy absorbed by the microorganisms and their resistance to UV. The resistance of different microorganisms to ultraviolet radiation varies considerably, in fact the most sensitive to treatment are vegetative bacteria followed by yeasts, bacterial spores, fungi and virus. It is evident from Table 1 that the UV dose required to inactivate viruses, yeasts, moulds and algae is much higher than for bacteria. It is considered that the absorption peak of UV-C is in the range 260-265 nm and these wavelengths are necessary for the inactivation of various microorganisms.

Table 1 Dosages required for microbial inactivation with UV-C light

Microorganism	Dose (J/m)
Bacteria (vegetative cell)	25
Viruses	45
Yeasts	66
Moulds	110
Algae	220
Bacteria (spore)	220
Bacillus subtilis (spore)	426

The main limit of this technology lies in poor penetration of UV-C light in food products, whether liquid or not. To be inactivated microorganisms should be directly exposed to radiation (López-Malo and Palou, 2005). In fact among the factors that can influence the efficacy of UV-C treatment there are the presence of other microflora (Wright et al., 2000), microorganism growth phase, species and number of cells, pH and product thickness. For this reason, application of UV-C light is particularly suitable for surface treatment of products where microbial and enzymatic activities mainly occur. Gram-negative bacteria *Pseudomonas, Escherichia* tend to be more susceptible to UV radiation than Gram-positive organisms *Baccilus*, *Staphylococcus* such that spore formers are more resistant than non-spore forming microorganisms (Jay, 1995). The appropriate UV-C treatment time for microbial inactivation could be during the lag phase, because the bacterial tend to be most resistant to UV radiation just after to cell division (Jay, 1995).

1.2.1.3 UV-light treatment: the application

Several works report the efficiency of UV light into the inactivation of different microorganisms. UV irradiation is mainly used in air and water purification but in the last

years it is potentially useful for milk and juice production (Koutchma et al., 2007; 2009; Matak et al., 2005).

Milk and dairy products contain high numbers of pathogens and spoilage microorganisms and thus, pose a greater challenge for UV treatment. Matak et al. (2005) achieved more than 5 log reductions of *Listeria monocytogenes, Cryptosporidium parvuum* and *E. coli* in goats' milk using the CiderSure 3500 UV apparatus with a two second UV treatment of 158 ± 16 J/m^2. The CiderSure 3500 apparatus allows the passage of fluid (in laminar flow) as a thin film on the UV exposed lamp, resulting in complete penetration of UV light into the fluid (Gómez-López et al., 2012). This condition is very important to increase the efficacy of penetration of UV light into the liquid.

Reinemann et al., (2006) reported a 2–3 log reduction in aerobic bacteria, yeasts and moulds, coliforms (including *E. coli*) and psychrotrophs in UV-treated milk at a dose of 1.5 kJ/l. The coliforms showed the greatest reduction while spore formers demonstrated the least reduction. Choudhary et al., 2011 examined the efficacy of a coiled tube UV reactor, for inactivation of *E. Coli* and *Bacillus cereus* spore in raw whole milk, pasteurized skimmed milk and soymilk using the total dose of 111.87 J/m^2 for a residence time of 11.3 seconds. The results showed more than a 7 log reduction of *E. coli* in skimmed milk, more than a 5 log reduction in soymilk and a 4 log reduction in raw whole milk. Gupta, 2011 reported a reductions of 7 log of total bacterial count in brine, sweet and acid whey, thus showing the potential use of UV light treatment in whey and brine in dairy processing. Orlowska et al., 2012 recently studied the effect of UV processing on the quality parameters of whole milk demonstrated that irradiated milk did not show any significant changes in the pH, viscosity, color and soluble solid contents. Bandla et al., 2012 investigated the reduction of the natural microorganisms of raw whole milk by UV treatment and the sensory and chemical properties of treated milk processed in a coiled tube UV reactor. The total UV dose for this treatment was 168.22 J/m^2 and the residence time of milk in the tube reactor was 17 seconds. This treatment reduced the standard plate count (SPC; an indicator of the natural microbial quality of milk) thus causing a 2.3 log reduction under these conditions.

UV light treatment can be used in other application. Disinfection using UV radiation is commonly used in wastewater treatment and is finding an increased usage in drinking

water treatment. New York City has approved the construction of a 2.2 billion US gallon per day ultraviolet drinking water disinfection facility, which must be online in 2012 (Trojan UV, 2012). For juice, the FDA requires the application of a Reduction Equivalent Dose (RED) of at least 400 J/m^2 at 253.7 nm (US-FDA, 2000). Otherwise, the phenomenon of photoreactivation may take place, which makes the organisms more resistant to UV-C than non-reactivated (Guerrero-Beltrán and Barbosa-Canovas, 2004). Basaran et al., 2004 investigated the influence of different apple cultivars upon the UV inactivation of *Escherichia coli* O157:H7 strains within unfiltered apple cider. The results of this study indicate that regardless of the apple cultivar used, a minimum 5-log reduction is achieved for all of the strains of *E. coli* O157:H7 tested. Franz et al., 2009 tested a potential use of a novel UV-C irradiation device in laboratory scale (Dean vortex technology) to inactivate bacteria in naturally cloudy apple juice. In this device, liquid flows through a helically wound tubing wrapped around a quartz glass tube containing a 9 W UV lamp with an irradiation intensity of 60 W/m^2 at 254 nm. The equipment was capable of reducing numbers of inoculated *Escherichia coli* and *Lactobacillus brevis* from an initial concentration of approximately 10^6 CFU/ml or 10^4 CFU/ml to below detectable limits in commercial naturally cloudy apple juice.

The effect of UV-C light was also investigated for microbial inactivation and quality control on fresh-cut fruits and vegetables. Fonseca and Rushing, 2006 evaluated the effect of UV-C light treatment on the microbial population and quality of watermelon. These results showed that exposing packaged watermelon cubes to UV light at 4.1 kJ/m^2 produced more than a 1 Log reduction in microbial populations without affecting juice leakage, color and overall visual quality. Manzocco et al., 2011 have studied the effect of UV-C light treatments at 1.2, 6.0, 12.0 and 24.0 kJ/m^2 with reference to germicidal efficiency and changes in fresh-like appearance of fresh-cut apple. Independently of UV-C light fluence, all treatments imparted the same germicidal effect with 1-2 log reduction in total viable counts. These results also demonstrated that UV-C light is an effective technology for food surface decontamination, but only if applied at mild intensity (1.2 kJ/m^2). Because, exceeded this intensity there are losses of compartmentalisation of surface apple cells, activation of dehydration and oxidative phenomena.

The use of different doses of UV-C radiation treatments (0, 2.4, 7.2, 12 and 24 kJ/m^2) to inhibit microbial growth of *Listeria monocytogenes, Salmonella enterica* and *Pseudomonas marginalis* (gram negative) was also tested (Escalona et al., 2010). These bacteria were studied under *in vitro* conditions and in baby spinach leaves. All radiation doses were effective in reducing bacterial growth, without affecting the sensory quality of fresh-cut baby spinach leaves. Guan et al., 2012 have evaluated the effect of UV-C light applied to both sides of mushrooms on microbial loads and product quality during cold storage. The microflora populations, color, antioxidant activity, total phenolics, ascorbic acid and the inactivation of *E. coli* O157:H7 were determined. The results of this study showed that UV-C radiation could potentially be used for sanitizing fresh button mushrooms and extending shelf-life. As a postharvest treatment on fresh produce, UV-C irradiation has been proven beneficial to reduce respiration rates, control rot development, and delay senescence and ripening in different fruits and vegetables such as apples, citrus, tomatoes, peaches, grapes, and guavas (de Capdeville et al., 2002; Jiang et al., 2010).

1.2.1.4 Advantages and limitation of UV technology

As was show in the previous sections the UV-C light treatment allows to be affective for microbial decontamination. Although it is also know that the antimicrobial effect of UV-C light would be limited to a very thin surface layer of the product.

The technology is easy to use and characterized by low maintenance, installation and operation cost with minimum energy usage. The treatment can be used for batch and continuous mode. UV-C systems can be operated automatically without special attention, what do not require highly specialized skills, and reinstallations are not highly demanding (Bintsis et al., 2000).

Another advantage of the UV treatment is that it does not produce any chemical residue and does not form toxic by-products (Keyser et al., 2008). In fact, it is used in water processing and to remove residual and disinfection by-products. This process works at ambient temperature with low detrimental effects on chemical components such as protein, color and flavor.

Despite the known germicidal efficiency of UV light, disinfection is not appropriate for treatment of products with high levels of suspended solids, turbidity, color, or soluble

organic matter (substances can react with UV radiation and also decrease the purification efficacy). Turbidity causes lower penetration of UV light and reduces the amount of microbial inactivation (Guneser and Karagul Yuceer, 2012). The conventional apparatus for UV treatment used in water treatment is not appropriate for pasteurization of milk or opaque juice. This problem can be solved with two approaches, the use of laminar flow and the use of turbulent flow that resulting in a better penetration of UV light. However UV processing was not completely studied and some effects on food matrix are still not well understood. UV light it is also dangerous to humans in fact, UV light damages human eyes, cause burns and skin cancer with prolonged exposure (Philips, 2006).

1.2.2 Pulsed light

1.2.2.1 Definition and general concept

Pulsed light (PL) is an emerging non-thermal technology, involves the use of high intensity pulsed broad spectrum light that flashes several times per second, typically 1-20 flashes per second (Dunn et al. 1995). The equipment used to produce PL is composed of one or more adjustable xenon lamp units, a power unit and a high voltage connection that allows the transfer of a high current electrical pulse. The light produced by the xenon lamp includes broad spectrum wavelengths from UV to near-infrared (Figure 3). The wavelength distribution ranges from 100 to 1.100 nm: UV (100-400 nm), visible light (400-700 nm), and infrared (700-1.100 nm).
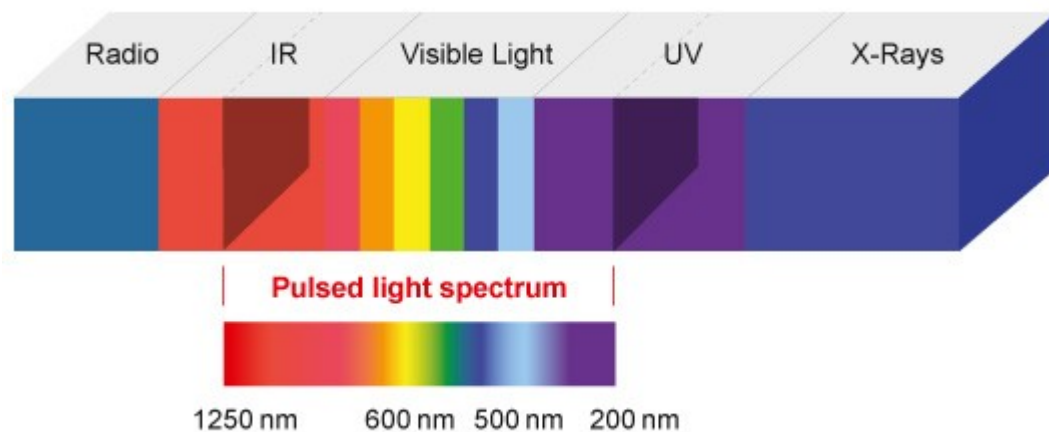
Figure 3 *Pulsed light electromagnetic spectrum.*

Pulsed light (PL) technology, is able to destroy microbes on the surfaces of dairy and other food products, food contact materials and medical devices and in liquid foods with significant rapidity and effectiveness(Krishnamurthy et al., 2008).

According to Wekhof (2000), the first works for microbial inactivation with flash lamps were performed in the late 1970s in Japan and the first work published in the scientific literature on the application of PL to inactivate microorganisms seems to be of Bank, et al., 1990.

Pulses of light used for food processing applications typically emit 1 to 20 flashes per second at an energy density in the range of about 0.01 to 50 J/cm^2 at the surface (Barbosa-Canovas et al. 1998).

PL technology has been used in the food industry for decontamination purposes after FDA approval in 1996. The regulatory conditions of PL technology for use in commercial food applications involve the use of a xenon lamp for emission of broad spectrum light at 200-1000 nm, with a pulse duration not exceeding 2 ms and the total treatment energy not exceeding 12 J/cm^2 (FDA, 2000).

The PL treatment dose is quantified by "fluence", which is total radiant energy of PL exposure on the food surface and the intensity is measured in J/cm^2 (Moraru, 2011). It is important highlight that the UV-C part of the spectrum, as described in the 1.1.1 section, is the most important for microbial inactivation. Furthermore, the inactivation efficacy of PL is influenced by most important factors such as the fluence incident on the samples, the distance of the samples from the light source, the sample thickness, the initial contamination levels and also food composition (Gómez-López et al., 2005 a; Sharma and Demirci, 2003; Hillegas and Demirci, 2003). The interaction of light and food is shown in figure 4.
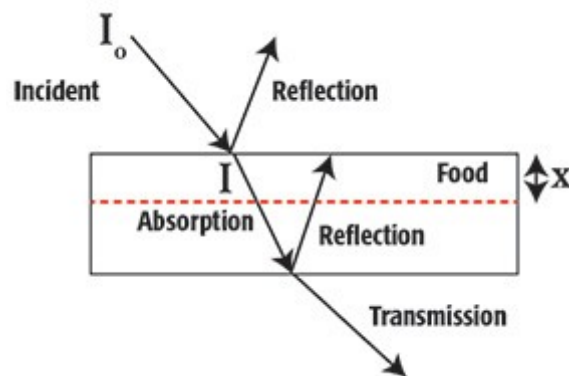
Figure 4 Interaction of light and food

1.2.2.2 PL treatment on microorganisms: mechanisms of action

Microbial inactivation by exposure to PL is attributed to the effect of the UV part of the spectrum and the energy density applied with the treatment, which in turn related with the pulse width and the high peak power of the pulse (Oms-Oliu et al., 2010 b). The mechanisms of inactivation by PL have similarities with UV light, which is primarily due to the formation of dimmers, resulting in distortion of the helix in microbial DNA. In addition, PL generates photothermal and photophysical effects causing rupture of the cell wall and membrane, which results in leakage of cytoplasm with eventual cell death (Oms-Olium et al., 2010; Miller et al., 2012).

The photothermal effects are based on the instantaneous heat generation inside the product due to the higher energy of PL and inactivation of bacteria due to UV absorption. In fact, in the absence of UV wavelengths, the light pulses are simply a fast method of transferring large amounts of thermal energy to the surface of the product (Mertens and Knorr 1992). Some authors have attributed the cell disruption to a photothermal effect caused by absorption of UV light when fluence, energy received by the sample, is excessive (Hiramoto et al., 1984; Wekhof et al., 2000; Wekhof et al. 2001).Wekhof et al., (2000), have noted that with an energy exceeding $0.5 \ J/cm^2$ the microbial inactivation is achieved through bacterial disruption during their temporary overheating, due to absorption of all UV light from a flash lamp. The water content of bacteria is vaporized, generating a small steam flow that induces membrane disruption (Takeshita et al., 2003). This overheating can be attributed to a difference in UV light absorption by bacteria and that of a surrounding medium (Elmnasser et al., 2007). In general, temperature increase of products exposed to PL is much lower and localized in a thinner surface layer than that of an equivalent continuous UV light treatment, due to the short duration of pulses (Oms-Oliu et al., 2010 b).

The photophysical effect occurs due to rapid release of intense energy in PL treatment and strikes membrane and cell composition. The impact of PL on proteins, membranes and other cellular materials probably coincides with the destruction of nucleic acids (Elmnasser et al., 2007). Takeshita et al., 2003 compared the inactivation of *Saccharomyces cerevisiae* cells by PL and classical continuous UV. They observed that the concentration of eluted

protein from yeast cells after pulsed light treatment was higher than observed under continuous UV treatment. This could indicate potential cell membrane damage induced by PL, with a distinct structural change in yeast cells (expanded vacuoles, cell membrane distortion). Instead, with continuous UV light, the yeast cell structure was almost similar to untreated cells.

Each microorganism has a different sensitivity to PL treatment. For example, Gram-positive bacteria, such as *B. cereus*, have been shown to be more resistant to the effects of PL than Gram-negative bacteria, such as *S. enteritidis* and *E. coli* (Anderson et al. 2000). The fungal spores, *A. niger* and *Fusarium culmorum*, have been shown a greater resistance to PL treatment compared with bacteria such as *E. coli*, *S. enteritidis*, or *B. cereus* (Anderson et al. 2000). According to these authors, the fungal spore resistance of *A. niger* could be attributed to the presence of protective dark pigments in the wall layers that surround the spore form. The variation in PL sensitivity may be related to differences in bacterial cell wall composition as well as due to their protective and repair mechanisms against the damage (Anderson et al. 2000).

Some examples of the effects of PL on some microorganisms *in-vitro* are shown in Table 2

Table 2 The effects of PL treatments on *in-vitro* microbial inactivation *(source Oms-Oliu et al., 2010 b)*

Microorganisms	Pulse energy (J)	Treatment time (µs)	Log reductions	Reference
Spoilage bacteria				
A. acidoterrestris	7	1.500	>5.2	Gómez-López et al., 2005 a
Lactobacillus sake	7	1.500	2.5	Gómez-López et al., 2005 a
L. mesenteroides	7	1.500	4.0	Gómez-López et al., 2005 a
P. aeruginosa	3	20	5.8	Rowan et al., 1999
P. fluorescens	7	1.500	4.2	Gómez-López et al., 2005 a
Pathogenic bacteria				
Bacillus cereus	3	20-1.500	4.9-3.0	Rowan et al., 1999; Gómez-López et al., 2005 a
C. perfringens	7	1.500	>2.9	Gómez-López et al., 2005 a

E. coli O157:H7	3-7	20-1.500	6.2-4.7	Rowan et al., 1999; Gómez-López et al., 2005 a
L. monocytogenes	3-7	20-1.500	4.4-2.8	Rowan et al., 1999; Gómez-López et al., 2005 a
Salmonella enteritidis	3	20	5.6	Rowan et al., 1999
S. typhimurium	7	1.500	3.2	Gómez-López et al., 2005 a
S. aureus	5.6	5.400	7.5-8.5	Krishnamurthy et al., 2004
Bacterial spores				
Bacillus cereus	7	1.500	>5.9	Gómez-López et al., 2005 a
Yeasts				
S. cerevisiae	3	20	4.9	Rowan et al., 1999
Fungal spores				
Aspergillus niger	1	1.000	4.8	Wekhof et al., 2001
Botrytis cinerea	7	1.500	1.2	Gómez-López et al., 2005 a

It is important to note that, unlike UV treatments, the PL-treated microorganisms do not show any enzymatic repair mechanism. The innate repair mechanisms of microorganisms may not be so effective in reversing the large amounts of damage incurred during PL treatment. This highlights the potential applications of PL treatment for food preservation (Rowan et al., 1999; Dunn, 2000).

1.2.2.3 Pulsed light treatment: the application

The microbial inactivation by PL treatment in several food, beverages and packaging materials have been studied.

Regarding liquid samples, Smith et al., (2002) in a pilot study investigated the inactivation of mesophilic aerobic bacteria in bulk tank milk with PL treatment by exposing 1 ml of milk to pulsed energy at 25 J/cm^2. PL treatment resulted in the complete elimination of the mesophilic bacteria.

The inactivation efficiency of PL treatment for *Staphylococcus aureus* in milk in a continuous mode was investigated by Krishnamurthy et al., (2007). The effects of parameters such as distance of milk sample from the PL source (5-11 cm), number of passes (1-3 passes) and flow rate of milk (20-40 ml/min) for PL treatment using 3 pulses per second and an exposed energy at 1.27 J/cm on milk samples per pulse were studied. The results of PL-treated milk showed the log reduction ranged from 0.55 to 7.26 log. The milk sample distance from the energy source was the statistically significant variable that influences the microbial inactivation. Choi et al., (2010) were the first to use PL treatment on infant formula as an alternative to conventional pasteurization. The batch mode was used on 2 mm thick infant food (infant beverage, infant meal and infant milk powder), containing 10^5 CFU/g of *Lysteria monocytogenes.* The inactivation of *L. monocytogenes* of infant formula by PL treatment was increased exponentially with the treatment period. The greatest cell inactivation was achieved in the infant beverage due to its low viscosity and turbidity compared to the infant meal and infant milk powder. Increasing the amount of solids will diminish the intensity of penetration of the UV radiation (Shama 1999; Bintsis et al., 2000).

Sonenshein (2003) investigated the inactivation effect of high intensity pulsed UV light (15.8 J/cm^2) on *Bacillus subtilis* spores suspended in sterile deionized water, where 3 pulses produced more than 6 log reduction. Water have the high degree of transparency to broad range of wavelengths including visible and UV light, for this reason increases the intensity of penetration of UV radiation and so the efficacy of PL treatment (Bintsis et al. 2000).

The decontamination effect of pulsed light on several minimally processed vegetables has also studied. Hoornstra et al., (2002) have demonstrated the microbial inactivation

naturally present on vegetable surfaces (white cabbage, leek, paprika, carrots and kale). The authors treated the samples with two pulses of wide spectrum PL that amounted to a fluence of 0.30 J/cm^2. The reduction in aerobic count at the surface of the vegetables varied from 1.6 log CFU/cm^2 for carrots to >2.6 log CFU/cm^2 for paprika. The efficacy of PL to inactivate vegetable spoilage microorganisms was again proved afterwards. Log reduction between 0.56 and 2.04 of mesophilic aerobic counts were achieved after treating with more than 2000 pulses several minimally processed vegetables (spinach, radicchio, lettuce, cabbage, carrot, green bell pepper) and soybean sprouts (Gómez- López et al., 2005b). The difference in log reduction between samples may be related to resistance of the natural microflora, the site of microorganisms in the samples (shadow effects) and protective substance in specific vegetables.

PL has also been studied to evaluate the surface disinfection of fresh fruits. Marquenie et al., (2003), found no inhibition of fungal development following the treatment of Botrytis cinerea inoculated on strawberries for up to 250 s and observed no effect on berry firmness. Literature data show that pulsed light can efficiently inactivate *Salmonella* spp., *Escherichia coli* and *Listeria innocua* in raspberries, strawberries, fresh-cut watermelon and mushrooms. Bialka and Demirci, (2008) have studied the application of pulsed UV-light on strawberries and raspberries at varying UV doses and times. On raspberries, maximum reductions of *Escherichia coli* O157:H7 and *Salmonella* were 3.9 and 3.4 log CFU/g at 72 and 59.2 J/cm^2, respectively. On the surfaces of strawberries maximum reductions were 2.1 and 2.8 log CFU/g at 25.7 and 34.2 J/cm^2, respectively. The effects of spectral ranges on inactivation of *Escherichia coli* and *Listeria innocua* in fresh-cut watermelon were determined by Ramos-Villaroel et al., 2012a. In this study was noted that the effectiveness of intense light pulses (ILP) was significantly affected when the UV-portion of light was blocked. The use of full spectrum treatments with a fluence of 12 J/cm^2, reduced *E. coli* population by more than 3 log cycles and the initial loads of *L. innocua* by 2.79l log. The same study was also performed to evaluate the bactericidal effect of PL by identifying the spectral range with antimicrobial activity and its effect on the quality of fresh-cut mushrooms (Ramos-Villaroel et al., 2012b).

Furthermore the effect of pulsed light at increasing fluence (17.5, 52.5, 105.0 and 157.5 kJ/m^2) was studied with reference to germicidal efficiency and changes in fresh-like appearance of sliced apple (Ignat et al., 2014). In this study, independently of fluence

viable count and inoculated bacteria were reduced by 1 and 3 log respectively. Fluence significantly affected weight loss, colour and sensory attributes of apple slices during storage at 6∘C. Exposure to excessive pulsed light fluence is associated with cell damage favouring browning reactions.

Besides its application on foods of vegetable origin, PL has been tested in a very limited number of other foods. Dunn et al., (1991) have evaluated the efficacy of PL treatment (fluence of 16 J/cm^2 and a pulse duration of 0.5 ms) on curds of commercially dried cottage cheese inoculated with *Pseudomonas* spp. In this case was observed a microbial reduction of 1.5 log cycle after 2 pulses. The surface temperature of the curd increased by 5 °C and the sensory evaluation showed promising results without any effects on the taste of the cheese. These results suggest a potential use of PL treatment on cheese curds to improve shelf-life of cottage cheese.

Hillegas and Demirci (2003) observed 0.97 log reduction for spores of *Clostridium sporogenes* inoculated in honey. The longer treatment time and the shorter distance between samples and lamp source, increase the inactivation but they also increase the samples temperature. Reducing the depth of honey an increase of the spore killing was observed, demonstrating that UV light has a limited penetration in the honey. The inactivation of *E. coli* O157:H7 and *L. monocytogenes* inoculated on raw salmon fillets by pulsed UV light was studied by Ozer & Demirci (2006). Two different surfaces were treated, the skin and the muscle, using a lamp that generated 5.6 J/cm^2 per pulse and 3 pulses per second. For *E. coli* O157:H7, the maximum log reduction was 1.09 on the muscle side and 0.86 on the skin side, while for *L. monocytogenes*, the reduction was 0.74 on the muscle side and 1.02 on the skin side.

1.2.2.4 Advantages and limitation of PL technology

Pulsed light technology with short pulse width and high dose, is a potential method for the treatment of foods that require a rapid disinfection (Wang et al., 2005). Other advantages of PL treatment are the lack of residual compounds and the absence of chemicals that can cause ecological problems and/or are potentially harmful to humans. Xenon flash lamps are also more environment friendly than UV lamps because they do not use mercury

(Gómez- López et al., 2007). Foods with smooth surfaces such as fresh whole fruit and vegetable, hard cheeses, or smooth surface meat slices are suitable for treatment with PL where the microbial contamination is on the surface of these foods (Oms-Oliu et al., 2010 b). Gómez-López et al. (2007) and Elmnasser et al. (2007) summarized the main limitations to the PL systems for food applications. The most important factor that limited the efficacy of this treatment is the sample heating. Hate can originate from the absorption of light by the food or lamp heating. Heating limited the treatment of alfalfa seeds (Sharma and Demirci 2003), grated carrots (Gómez-López et al. 2005b), and raw salmon fillets (Ozer and Demirci 2006). Food composition also limited the efficacy of PL. In fact, high-protein or oily foods are not suitable for decontamination by intense light pulses, because part of the radiation is absorbed by proteins and oils (Roberts and Hope 2003). Regarding solid and fluid opaque foods the decontamination efficacy is limited to a surface layer due to absorption of the light. The effect of product thickness on PL-induced microbial inactivation was studied by Hillegas and Demirci (2003). They have observed a different reduction of *Clostridium sporogenes* in two different thickness of honey samples (2-mm and 8-mm), with absence of microbial reduction in 8-mm thickness of samples. Furthermore, the distance from the light source influences the inactivation efficacy of PL: the longer the distance between the sample and the lamp, the lower the lethality of the process (Gómez-López et al., 2005a). The inactivation efficacy is markedly reduced when the initial microbial contamination is very high Gómez-López et al., (2005b) have observed a strong decrease in inactivation efficiency when high counts of *L. monocytogenes* were reached on the surface of an agar medium. The less efficient decontamination is due to the shadow effect. The shading effect will reduced the effective radiation dose available for microbial inactivation. The shadow effect can also occur in foods with rough or uneven surfaces, or pores resulting in a reduction of treatment effectiveness, because the microorganisms are able to fit into small openings and have a protective effect (Lagunas-Solar et al., 2006). PL is safe to apply but some precautions have to be taken to avoid exposure of workers to light and to evacuate the ozone generated by the shorter UV wavelengths (Gómez-López et al., 2007).

1.2.3 Ultrasound

1.2.3.1 Definition and general concept

Ultrasound is defined as sound waves having frequency that exceeds the hearing limit of the human ear (~20 kHz). Ultrasound is one of the emerging technologies that were developed to minimize processing, maximize quality and ensure the safety of food products.

Ultrasound is applied to impart positive effects in food processing such as improvement in mass transfer, food preservation, assistance of thermal treatments and manipulation of texture and food analysis (Knorr et al., 2011).

Based on frequency range, the applications of ultrasound in food processing, analysis and quality control can be divided into low and high energy.

Low energy (low power, low intensity) ultrasound has frequencies higher than 100 kHz at intensities below 1 W/cm^2, which can be utilized for non-invasive analysis and monitoring of various food materials during processing and storage to ensure high quality and safety. Low-intensity ultrasound is most commonly applied as an analytical technique to give information on the physicochemical properties of food such as firmness, ripeness, sugar content, acidity, etc (Demirdŏven & Baysal, 2009).

High energy (high power, high-intensity) ultrasound uses intensities higher than 1 W/cm^2 at frequencies between 20 and 500 kHz, which are disruptive and induce effects on the physical, mechanical or chemical/biochemical properties of foods (McClements, 1995). These effects are promising in food processing, preservation and safety. High-intensity ultrasound was used for many years to generate emulsions, disrupt cells and disperse aggregated materials, but recently it is used for crystallization processes, degassing of liquid foods, enzyme inactivation to enhance shelf-life and quality food products, microbial inactivation and induction of oxidation reactions (Knorr et al., 2004; Zheng and Sun, 2006; Awad et al., 2012).

The ultrasound waves have characteristic wavelength, velocity, frequency, pressure and period. The interaction of sound waves with matter alters both the velocity and attenuation of the sound-waves via absorption and/or scattering mechanisms, that it is correlate with many physicochemical properties of materials (McClements, 2005; Buckin et al., 2002). The velocity of sound is the product of frequency and wavelength, thereby high frequency sound waves have short wavelength while low frequency waves have long wavelength.

In general, energy, intensity, pressure, velocity and temperature are the main parameters affecting power ultrasound. High power ultrasound can be described by Patist & Bates, 2008:

$$P_a = P_{a\,max} \cdot \sin(2\pi ft). \qquad\qquad \text{(Eq. 3)}$$

P_a is the acoustic pressure (a sinusoidal wave), which is dependent on time (t), frequency (f) and the maximum pressure amplitude of the wave (Muthukumaran et al., 2006). $P_{a\,max}$ is related to the power input or intensity (I) of the transducer:

$$I = P_{a\,max}/2\rho v \qquad\qquad \text{(Eq. 4)}$$

where ρ is the density of the medium and v is the sound velocity in the medium.

With low intensities (or high frequencies), acoustic streaming is the main mechanism (Leighton, 1994; Leighton, 2007). Acoustic streaming is the motion and mixing within the fluid without formation of bubbles (Alzamora, et al., 2011). Higher intensities (low frequencies) induce acoustic cavitation (Mason, 1998) due to the generation, growth and collapse of large bubbles, which causes the liberation of higher energies (Alzamora et al., 2011).

Ultrasound can be used for food preservation in combination with other treatments by improving its inactivation efficacy. The ultrasonication used alone, is the application of ultrasound at low temperature, it can be used for the heat sensible products. It requires long treatment time to inactivate enzymes and microorganisms. During the application there may involve a temperature increase depending on the ultrasonic power and treatment time (Zheng and Sun, 2006; Ercan and Soysal 2013).

Instead, thermosonication is a combined method of ultrasound and heat. The product is subjected to ultrasound and moderate heat simultaneously. This method produces a greater effect on inactivation of microorganisms. When it is used for pasteurization or sterilization, lower process temperatures and treatment times are required, to obtained the same lethality values as with conventional processes (Şahin Ercan and Soysal 2013; Mason et al., 1996; Villamiel et al., 1999).

1.2.3.2 Ultrasound treatment on microorganisms: mechanisms of action

The potential use of ultrasound as microbial decontamination technique was well documented (Cameron et al., 2009; Drakopoullou et al., 2009) even though its application to food is relatively recent. It appears to have a low lethality at low temperature but in combination with pressure (manosonication), heat (thermosonication) or both (manothermosonication), it can improve the microbial inactivation efficacy (Adekunte et al., 2010; Cameron et al., 2009; Gera & Doores, 2011; Manas et al., 2000a; Raso et al., 1998a).

Many researches have been done to understand the microbial inactivation mechanisms of ultrasound that it was simply explained with acoustic cavitation phenomena and its physical, mechanical and chemical effects (Piyasena et al., 2003; Şahin Ercan and Soysal 2011). The mechanism of microbial killing is mainly due to thinning of cell membranes, localized heating and DNA damage via free radical production (Earnshaw et al., 1995, 1998; Butz and Tauscher, 2002; Fellows, 2000). This phenomena was simply represented in figure 5.
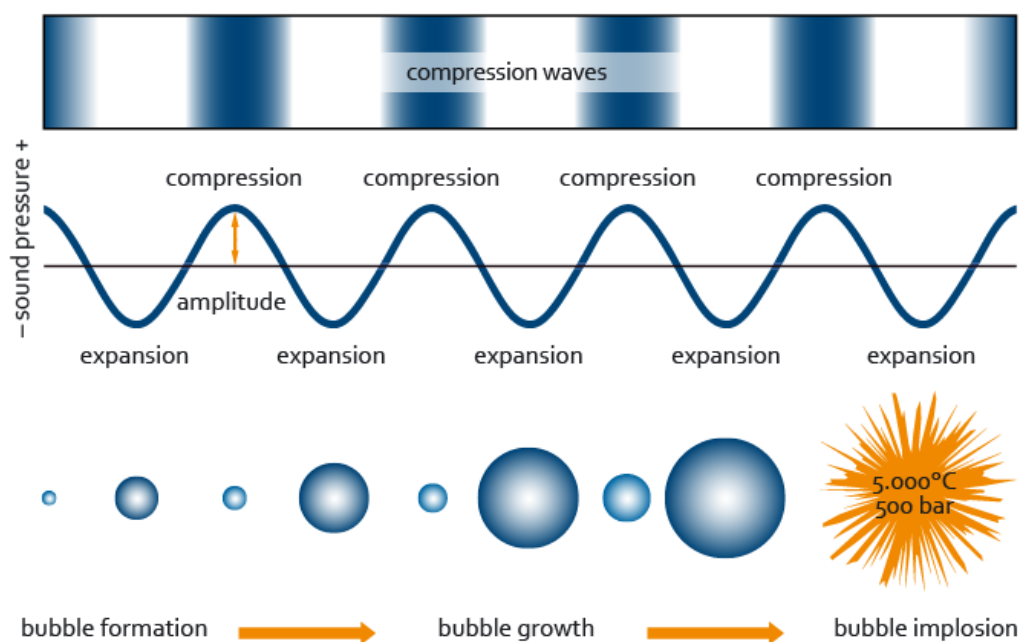
Figure 5 *Ultrasound Cavitation.*

During the sonication process, longitudinal waves are created when a sonic wave meets a liquid medium, thereby creating regions of alternating compression and expansion (Sala et al., 1995). With these changes of pressure the cavitation occurs and gas bubbles are formed in the medium. These bubbles have a larger surface area during the expansion cycle, which increases the diffusion of gas, causing the bubble expansion. A point is reached where the ultrasonic energy provided is not sufficient to retain the vapour phase in the bubble; therefore, rapid condensation occurs. The molecules collide violently, creating shock waves and these create regions of very high temperature and pressure, reaching up to 5000 °C and 500 bar. The pressure changes resulting from these implosions are the main bactericidal effect in ultrasound (Earnshaw, 1998; Piyasena et al.,2003).

The inactivation efficiency varying greatly depending on the bacterial species and growth medium (Sala et al., 1995). Different kinds of microorganisms have different membrane structure. Such as, Gram-positive and Gram-negative bacteria do not show same behavior against ultrasonic waves due to their different cell and membrane structures. Gram-positive bacteria have a thicker cell wall and lack of membrane and also Gram-negative bacteria have a thinner cell wall with an outer membrane (Piyasena et al., 2003). Furthermore bacterial spores are far more resistant to sonication than vegetative cells, thus extend the

periods of ultrasonication would be required to make a product safe (Feng et al., 2008). Drakopoulou et al., 2009 examined the disinfection efficacy of ultrasound irradiation on different bacteria groups, namely total coliforms (TC), faecal coliforms (FC), *Pseudomonas* spp. (PS), faecal streptococci (FS) and *Clostridium perfringens* species (CP), found in wastewaters. They reported that Gram-negative bacteria are more readily susceptible to ultrasound inactivation than the Gram-positive ones. This observation is in accordance with a study on efficacy of ultrasonic treatment in a continuous flow process to inactivate total bacteria in milk and *P. fluorescens* and *Streptococcus thermophilus* in tryptic soy broth (Villamiel and de Jong, 2000). In which was noticed that gram-negative bacteria (*P. fluorescens*) were more susceptible to the ultrasonic treatment than gram-positive bacteria (*S. thermophilus*). In addition, the microbial inactivation also varies among different strains. For example, *Escherichia coli* and *Saccharmyces cerevisiae* were reduced by more than 99% after ultrasonication, whereas *Lactobacillus acidphilus* was reduced by 72% and 84% depending on the media used (Cameron et al., 2008).

Other factors that are known to affect the effectiveness of microbial inactivation are amplitude of the ultrasonic waves, exposure/contact time, volume of food being processed, the composition of the food and the treatment temperature (USDA, 2000). Effect of ultrasound on microbial inactivation also depends on intensity and frequency of ultrasound applied. Generally, frequency range of 200-600 kHz enhanced the effects of ultrasound on microorganisms. Wordon, et al. (2011) suggested that high frequency of ultrasound was more effective in irradiation of microorganisms.

1.2.3.3 Ultrasound: the application

Much of the ultrasonic work showing microbial inactivation has been studied in a variety of media with only a few studies focused on milk and the energy required for microbial inactivation in these studies was high. Cameron et al., 2009 evaluated the effect of sonication in raw and pasteurized milk. In this study the efficiency of sonication occurred between 6 and 10 minutes of batch treatment at 20 kHz, and it was effective without the use of heat, against spoilage microorganisms and potential pathogens including *E. Coli*, *Pseudomonas fluorescens* and *Listeria monocytogenes*. *Listeria monocytogenes* and *E. Coli* were also thermosonicated in milk by others (Earnshaw et al., 1995; Zenker et al.,

2003; Gera and Doores, 2011). Gera and Doores (2011) showed that pulsed sonication at 24 kHz causes mechanical damage to the bacterial cell wall and cell membrane when treated at temperatures between 30 and 35 °C. But they have also noticed that milk had a microbial protective effect, with lactose exerting a positive effect on bacterial survival. In a separate study Bermudez-Aguirre et al., (2009a; 2009b) evaluated the synergistic effect of heat (63°C) combined with sonication (24 kHz) used to inactivate *Listeria innocua* and reduce the mesophilic bacteria count in raw whole milk. In this study there was a reduction by 0.69 log after 10 minutes and 5.3 log after 30 minutes, resulting in an extension of shelf-life. Thermosonication at 20 kHz and temperatures up to 50 °C were used to inactivate *Cronobacter sakazakii* and reduce the microbial count in reconstituted infant formula by up to 7.04 log units after 2.5 minutes of treatment (Adekunte et al., 2010). Furthermore, thermosonicated milk was used to make a soft type of fresh cheese and after 23 days of refrigerated storage the microbial counts remained low (mesophilic, 4 log; psychrophilic, 3.5 log; Enterobacteriaceae, 3 log) (Bermudez-Aguirre and Barbosa-Canovas, 2010). Juraga, et al., (2011) work with high intensity ultrasound to investigate inactivation of Enterobacteriaceae in raw milk. For ultrasounds treatment, they used three parameters: temperature (20, 40 and 60°C), amplitude (120, 90 and 60 μm) and time (6, 9 and 12 min). They noticed that inactivation of microorganisms depends on the amplitude of the ultrasonic waves, the exposure/contact time with the microorganisms and the temperature of treatment. The achieved results indicate significant inactivation of microorganisms under longer period of treatments with ultrasonic probe particularly in combination with higher temperature and amplitude.

Microbial inactivation using ultrasound has been also investigated in other foods. Levels of *E. coli* O157:H7 were reduced by 5 log cfu/mL with ultrasound in apple cider and the inactivation of *E. coli* K12 was enhanced using ultrasound at ambient temperatures (D'Amico et al., 2006). The application of different ultrasonic treatments to fresh orange juice and the comparison of this stabilization method with the conventional thermal processes was evaluated by Valero et al., (2007). Arroyo et al., (2012) have examined the efficacy of ultrasound under pressure treatments combined with heat for the inactivation of *C. sakazakii* inoculated in apple juice. Below 45 °C, the inactivation by ultrasound under pressure was independent of temperature. Above 64 °C, the lethal effect of ultrasound under pressure was negligible when compared to the lethality of the heat treatment at the

same temperature. Between 45 °C and 64 °C, the lethality of the combined process (manothermosonication) was higher, a synergistic effect was observed.

High power ultrasound is reported as a potential tool to reduce microorganisms in poultry (Feng and Yang, 2011; Haughton et al., 2012; Loretz et al., 2010; Smith, 2011), meat (Dolatowski and Stasiak, 2002), and pork (Birk and Knøchel, 2009; Morild et al., 2011). As reported above, the microbial inactivation efficacy of ultrasound increase in combination with other methods such as pressure, heat, steam and other physical treatments (Haughton et al., 2012; Pagan et al., 1999; Raso et al., 1998a). The combined heat/steam application with ultrasound was accomplished with a steam–ultrasound technique by allowing the steam to reach the microorganisms in the microstructure and cavities on the meat surface more efficiently. This synergistic effect of ultrasound was used to inactivate *Campylobacter, Enterococcus faecium,* and *Bacillus subtilis* (Boysen and Rosenquist, 2009; Haughton et al., 2012; Mason, 1998; Pagan et al., 1999; Piyasena et al., 2003; Raso et al., 1998). Musavian et al. (2014) used an ultrasound and steam combination (sonosteam) at 90-94 °C and 30-40 kHz on naturally *Campylobacter* contaminated broiler carcasses. The results obtained in this study suggest that the combined steam-ultrasound treatment can be significantly reduce the number of *Campylobacter* (reduction of ultrasound was between 1-1.37 log). Similarly, Morild et al. (2011) reported that steam (130 °C/3.5-5 atm) and ultrasound (30-40 kHz) treatments for 0.5, 1.0, 1.5, and 2.0 s on the skin and meat surfaces of pork inoculated at two levels significantly reduced *Salmonella typhimurium*, *Y. enterocolitica*, and *E. coli.*

Furthermore, it has been proven that ultrasound is an effective method in the inactivation of enzymes when it is used alone or with temperature and pressure. There are many enzymes inactivated with ultrasound such as glucose oxidase (Guiseppi-Elie et al., 2009), peroxidase (Şahin Ercan and Soysal 2011; De Gennaro et al., 1999), pectin methyl esterase (Raviyan et al., 2005), protease and lipase (Vercet et al., 2001), watercress peroxidase (Cruz et al., 2006) and polyphenoloxidase (Raso and Barbosa-Canovas 2003).

Ultrasonic technologies could have a strong presence in the future of the food industry, the combination of ultrasound and pressure and/or heat it has proved very promising.

1.2.3.4 Advantages and limitation of Ultrasound technology

Ultrasound technology has been used as a fast, reliable, relatively cheap, and simple alternative application to conventional food processing. Ultrasonic waves are safe and non-toxic; these advantages have gained acceptance of ultrasound over other antimicrobial methods (Kentish and Ashokkumar, 2011). The combination of ultrasound and/or physical-biological methods contributes to the enhancement of microbial inactivation and elimination.

Ultrasound processing in combination with lower heat process temperatures has many advantages. It can lead to better quality products, with improvements in taste, texture and appearance. It also could result in reduced energy requirements and therefore reduced cost. The application of ultrasound with heat will require the design of new types of processing equipment and this is not easy. Even though this technology is very practical and has greater homogeneity as well as significant energy savings; more research is still needed to be carried out and effective ultrasonic systems should be designed which support large scale operations to be adapted to various food systems (Awad et al., 2012; Chemat et al., 2011; Gallego-Juárez et al., 2007; Knorr et al., 2004; Lee and Feng, 2011; Lillard, 1994; Piyasena et al., 2003; Rastogi, 2010)

The thermosonication is particularly suited to liquids and liquids containing solids which could be processed with a continuous flow arrangement with clean or aseptic filling. Furthermore, ultrasound is a "green" technologies and it needs adequate validation to establish the microbial and enzymatic inactivation efficacy. The prospect of applying ultrasound to foods is conceptually possible but it is important to consider in detail the factors that may influence the efficacy, also in terms of ultrasound penetration. Like all innovative technologies, further studies are necessary for the commercial adaptation of ultrasound technology in the food industry.

1.2.4 Ionizing radiation

1.2.4.1 Definition and general concept

Ionizing radiation corresponds to electromagnetic radiation or particulate energy associated with a greater than 10 eV (electron Volt). Below this value of energy, radiation is "non-ionizing" and there class including radiation, infrared, ultraviolet, or electromagnetic fields of extremely low frequency (microwave). With the energy that is associated with ionizing radiation are capable of moving the electrons of atoms and molecules and converting them into ions, hence the term "ionizing radiation" (Figure 6).

Ionizing radiations are applied to foods to improve their keeping quality. Foods treated with ionizing radiation are known as "irradiated". They are not "radioactive".
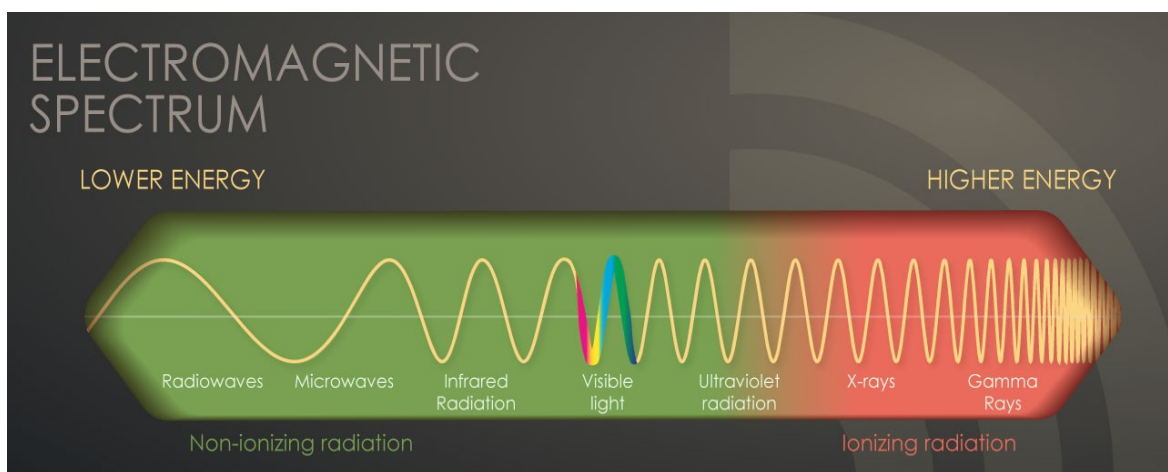
Figure 6 Electromagnetic spectrum and ionizing radiation.

According to the Codex General Standard for Irradiated Foods, ionizing radiations recommended for use in food processing are:

1. **Gamma ray:** Gamma-rays are produced by radioactive isotopes (radionuclides), which is either cobalt-60 or cesium-137. Both cobalt-60 and cesium-137 emit highly penetrating gamma rays that can be used to treat food in bulk or in its final packaging. Cobalt-60 is, at present, the radioisotope most extensively employed for

gamma irradiation of food (Steward, 2004; Liberty et al., 2013). Radioactive substances emit gamma rays all the time. When not in use, the gamma ray "source" is stored in a pool of water which absorbs the radiation harmlessly and completely. To irradiate food or some other product, the source is pulled out of the water into a chamber with massive concrete walls that keep any rays from escaping.

2. **X-rays:** X-rays are produced by aircraft operating at an energy level equal to or less than 5 MeV. X-rays have been shown to be more penetrating (20 cm) than electron-beam. To produce the X-rays, a beam of electrons is directed at a thin plate of gold or other metal, producing a stream of X-rays. X-rays can pass through thick foods, and require heavy shielding for safety. However, the machine can be switched on and off, and no radioactive substances are involved (Liberty et al., 2013).

3. **Accelerated electrons (electron-beam):** The electrons are accelerated systems obtained by operating at a level of energy equal to or less than 10 MeV. A major advantage of machine-sourced ionizing radiation is that no radioactive substance is involved in the whole processing system. Powered by electricity, electron-beam machines use linear accelerators to produce accelerating electron beams to near the speed of light. The electron beam generator can be simply switched on or off. There are no radioactive materials in the process. The high energy electron beams have limited penetration power (only to a depth of three centimeters) and are suitable only for foods relatively thin (Steward, 2004; Liberty et al., 2013).

Food irradiation is not new; interest was shown in Germany in 1896 and it began in the early 1920s, while in the 1950/60s the US Army Natick Soldier Center (NATICK) experimented with both low dose and high dose irradiation for military rations (Steward, 2004; Liberty et al., 2013).

The Joint FAO (Food and Agriculture Organization)/IAEA (International Agency for Atomic Energy)/WHO (World Health Organization) Expert Committee on the Wholesomeness of Irradiated Food (JECFI), evaluated the results (extensive toxicological studies) obtained in different international projects. This Committee concluded in 1980 that the irradiation of any food commodity up to an overall average dose of 10 kGy presented no toxicological hazard and no special nutritional or microbiological problems (WHO, 1981; Diehl, 2002). After, in 1997, these agencies have concluded that no maximum dose should be accepted. However, the Codex General Standard for irradiated foods (CODEX STAN 106-1983, REV. 1-2003) states that the maximum absorbed dose for a food must not exceed 10 kGy.

Irradiation is extensively used in the medical field for sterilizing instruments, dressings etc. Food irradiation by ionizing radiation is the process in order to control food-borne pathogens, reduce microbial load and insect infestation, inhibit the germination of root crops, and extend the durable life of perishable produce (International Consultative Group, 1991).

The International Atomic Energy Agency (IAEA) has reported that more than 50 countries have approved the use of irradiation for about 50 different types of food, and 33 are using the technology commercially. The positive list of irradiated products varies between countries but is often limited to spices, herbs, seasonings, some fresh or dried fruits and vegetables, seafood, meat and meat products, poultry and egg products (Liberty et al., 2013).

The irradiation dose applied to a food product is measured in terms of kilograys (kGy). One kilogray is equivalent to 1.000 grays (Gy), 0.1 megarad (Mrad), or 100,000 rads. The basic unit is the gray, which is the amount of irradiation energy that 1 kilogram of food receives.

The dose rate is determined by the dose absorbed by the food, per unit of exposure time. This is the power that can be expressed in kilowatts per kilogram of food or kGy per unit time.

The irradiation dose applied to a food product will depend upon the composition of the food, the degree of perishability, and the potential to harbor harmful microorganisms. The amount of radiation that a food product absorbs is measured by a dosimeter (Roberts et al., 1995; Liberty et al., 2013).

Public opinion about food irradiation was generally positive during the growth period of nuclear technology in the 1950s and 1960s, but with the advance of the antinuclear movement since the 1970s the climate of opinion changed and opposition to the practical use of food irradiation has grown (Diehl, 2002). In many countries (USA, China, Brazil, South Africa, Vietnam and Japan) the food irradiation is practiced to some extent, on the contrary the status of food irradiation in the EU is not very encouraging, this country such as some others are reluctant to use the ionizing radiations for food preservation (Diehl, 2002).

1.2.4.2 Ionizing radiation treatment on microorganisms: mechanisms of action

Ionizing radiations inactivate microorganisms by damaging critical elements in the cell, most often the genetic material. This damage prevents multiplication and also randomly terminates most cell functions. Damage to genetic materials occurs as a result of a direct collision between the radiation energy and genetic material, or as a result of the radiation ionizing an adjacent molecule (in most cells, is usually water), which in turn reacts with the genetic material (Figure 7) (Grecz et al., 1983).

In the first instance, an electron randomly strikes the genetic material of the cell and causes a lesion in the DNA. The lesion can be break in a single strand of the DNA or, if the orientation of the DNA is appropriate, the energy or electron can break both strands on the DNA. Single-strand lesions may not be lethal in and of themselves, and may in fact results in mutations. However, large numbers of single-strand lesions may exceed the bacterium's repair capability, which ultimately results in the death of the cell (RA Molins 2001). A double-strand lesion occurs when the electron strikes adjacent areas on both strands of DNA. This in effect divides the DNA into two pieces. Double-strand lesions are almost invariably lethal. However, due to the particularly orientation of the DNA in relation to the

irradiation source, double-strand lesions occur much less frequently than do single-strand lesions.

The interactions of radiation with molecules adjacent to the genetic material are more complex. Radiation causes water molecules to lose an electron, producing H_2O^+ and e^-. These products react with other water molecules to produce a number of compounds, including hydroxyl radicals (OH^-) and hydrogen peroxide (H_2O_2) (Arena 1971). These molecules react with the nucleic acids and the chemical bonds. As with the direct interaction of radiation with DNA, the indirect action can result in both single- and double-strand lesions, with the same overall effects (Molins, 2001).
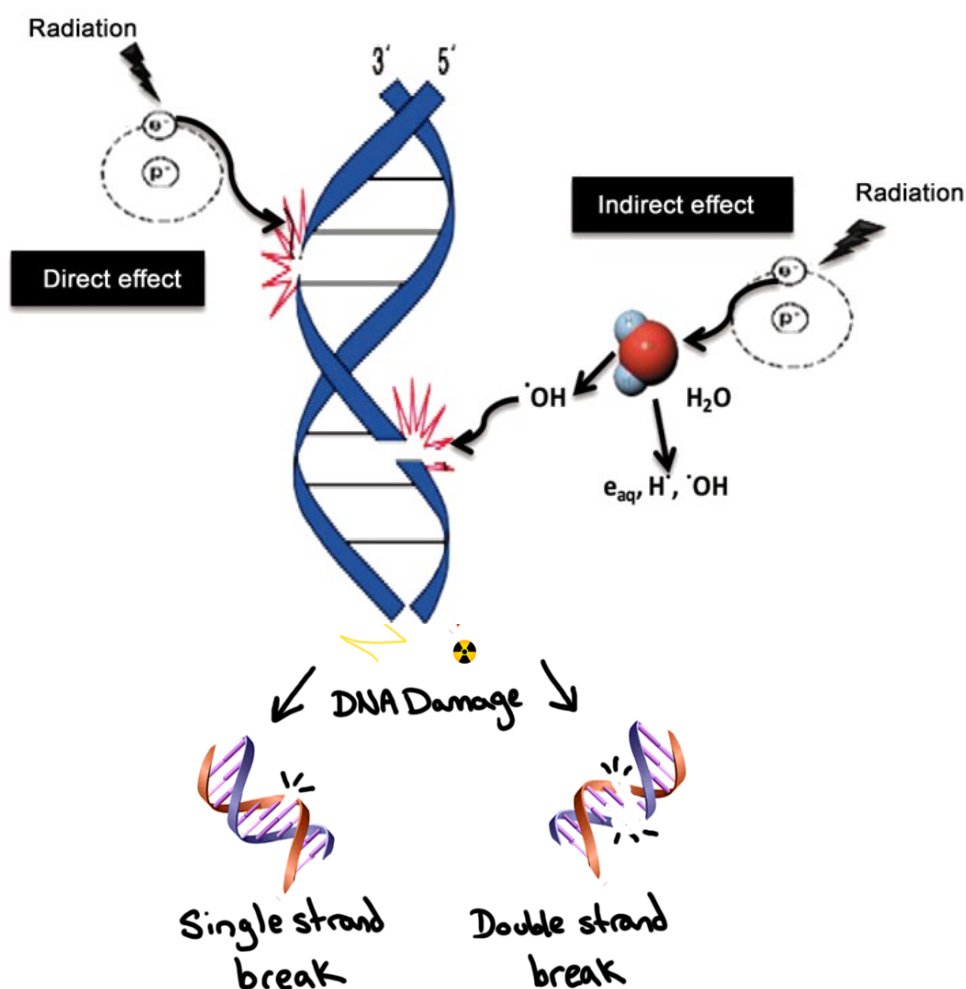
Figure 7 *Damage to genetic materials, direct and indirect effects.*

In addition to effect on genetic material, radiation has a variety of effects on the other components of the cell. Applying radiation to a cell results in the direct and indirect interaction with cell components such as membranes, enzymes and plasmids. These interactions may have a role in the survival of sub-lethally injured bacteria, in a cell that has not sustained lethal genetic damage may be damaged in other ways that complicate or impede survival of the injured cell (Molins, 2001).

A large amount of data is available on the sensitivity of microorganisms to irradiation processing; this varies greatly from micro-organism to micro-organism and is also dependent on other extrinsic factors. Vegetative cells are less resistant to irradiation than spores, whereas moulds have a susceptibility to irradiation similar to that of vegetative cells. However some fungi can be as resistant as bacterial spores (Farkas, 2006). Compared to bacteria, viruses generally require higher radiation doses for inactivation (Crawford et al., 1996). Studies have shown that irradiation doses of 2 and 3 kGy destroyed *Yersinia* spp. and *Listeria* spp., respectively, with the microorganisms being undetectable during storage of irradiated fish (Montgomery et al., 2003). Irradiation (1, 2, and 3 kGy) significantly improved the microbiological quality of the chicken by reducing the total bacterial count (TBC), with the decrease in TBC being dose dependent. In all the irradiated samples, no fecal coliforms were detected (Kanatt et al., 2005).

The efficacy of ionizing radiation for microbial inactivation depends mainly on the dose and the level of resistance of the microorganisms. Other factors such as temperature, pH, presence of oxygen and solute concentration have also been shown to be correlated with the radiolytic products formed during irradiation which affect the effectiveness of ionizing radiation (Liberty et al., 2013).

1.2.4.3 Ionizing radiation: the application

Ionizing radiations have been demonstrated to be an effective means of destroying both pathogenic and nonpathogenic bacteria, as well as parasites and to a lesser degree, viruses. As X-ray irradiation does not produce radioactive waste and can pass through thick materials (approximately 30-40 cm), it can be applied as a more efficient preservation method for foods to achieve microbial decontamination (Oner and Wall, 2013).

During the past 60 years, the irradiation of the raw materials such as tuber and bulb crops, stored grains, dried ingredients, meats, poultry and fish, or fruits has an enormous literature (Wilkinson & Gould, 1996; Farkas 1999; Molins, 2001).

Previous studies have demonstrated that X-rays can result in very high microbial reduction efficacy for different pathogens. The irradiation treatment of frozen poultry with a dose of 3-5 kGy, and 1.5-2.5 kGy for chilled poultry were effective to reduce the most resistant serotype of *Salmonella* and *Campylobacter*, with about 3 log-cycles of redrution (Kampelmacher, 1984). Serrano et al., (1997) on the basis of their study have evaluated that a minimal dose of 0.5 kGy would be sufficient to eliminate *S. enteritidis* from the surface of whole eggs. A dose of 1.5 kGy would be sufficient to eliminate the organism from whole shell eggs and liquid whole eggs without affecting the egg quality.

Van Calenberg et al., (1998) have examined the effect of irradiation with X-rays and electron beams on the microbiological quality of white pepper, sweet red paprika and nutmeg. The results on coliforms, total mesophilic counts and thermophilic spores after irradiation with doses ranging from 0 to 10 kGy showed that for the selected dry spices no major differences between the two irradiation techniques were found. Reduction of the microorganisms through irradiation was effective. After, other study was carried out on the effect of irradiation with X-rays and e-beams on the microbiological quality of minced chicken breast meat, shrimps and strawberries. Samples were irradiated with doses ranging from 0 to 1.5 kGy. The results confirm that irradiation below 2 kGy significantly reduced the natural microflora of the tested foodstuffs (Van Calenberg et al., 1999).

More recently was also demonstrated the efficacy of X-ray for microbial inactivation. Mahmoud, (2009a), has evaluated the inactivation effect of X-ray treatments on *Escherichia coli* O157: H7, *Salmonella enteric*, *Shigella flexneri* and *Vibrio parahaemolyticus* inoculated in ready-to-eat shrimp. The inoculated shrimp samples were placed in sterilized bags and treated with different doses of X-ray (0.1, 0.2, 0.3, 0.5, 0.75, 1.0, 2.0, 3.0 and 4.0 kGy) at ambient temperature. In this study more than a 6 log reduction of *E. coli* O157: H7, *S. enterica*, *S. flexneri* and *V. parahaemolyticus* was achieved with 2.0, 3.0, and 4.0 kGy, respectively.

The inactivation efficacy of X-ray treatments on *Cronobacter* (*E. sakazakii*) in tryptic soy broth (TSB), skim milk (0% fat), low-fat milk (1% and 2%) and whole-fat milk (3 and 5 %) was also evaluated (Mahmoud, 2009 b). The samples were inoculated with the *Cronobacter* and were treated with different X-ray doses (0.1, 0.5, 0.75, 1.0, 2.0, 3.0, 4.0, 5.0 and 6.0 kGy). Also in this case, for all treated samples, a greater than 7 log reduction in *Cronobacter* population was observed with 4.0, 5.0 and 6.0 kGy, respectively.

A similar study was carried out on spinach leaves and tomatoes (Mahmoud, et al., 2010; Mahmoud, 2010). The Inactivation of inoculated (approximately 8-9 log/ml) *E. coli* O157:H7, *L. monocytogenes*, *S. enterica* and *S. flexneri* on spinach leaves using X-ray (0.1, 0.2, 0.3, 0.5, 0.75, 1.0, 1.5 and 2.0 kGy) and on tomatoes using X-ray (0.1, 0.5, 0.75, 1.0, and 1.5 kGy) were studied. For all tested pathogens more than a 5 log reduction was achieved in spinach leaves with 2.0 kGy. While approximately 4.2, 2.3, 3.7 and 3.6 log reduction of *E. coli* O157:H7, *L. monocytogenes*, *S. enterica* and *S. flexneri* per tomato were achieved by treatment with 0.75 kGy, respectively. Instead more than a 5 log reduction per tomato was achieved at 1.0 or 1.5 kGy for all tested pathogens.

Mahmoud et al., 2012 have also determined the efficacy of X-ray processes in inactivating *L. monocytogenes* levels in smoked catfish during storage at 5°C for up to 5 weeks. Smoked catfish fillets inoculated with *L. monocytogenes* were treated with 0.0-2.0 kGy and stored at 5°C for 5 weeks. The results of this study indicate that X-ray at 2.0 kGy can eliminate *L. monocytogenes* (below the detection limit) and extend the shelf-life (until 35 days) of smoked catfish stored at refrigeration temperature.

The effect of X-ray irradiation on the quality of fresh-cut, refrigerated purple-fleshed sweet potato cubes was investigated (Oner and Wall, 2013). Packaged sweet potato cubes were treated with 0, 0.25, 0.5, 0.75 or 1 kGy and stored at 4 °C for 14 day. In this study was observed that after 14 days, total aerobic bacteria counts were 4.1 and 3.2 log/g, and mould–yeast counts were 3.3 and 3.0 log/g in treated samples (0.75 and 1 kGy), respectively.

The effectiveness of electron beam irradiation on the shelf-life of mozzarella cheese was also evaluated using five irradiation doses (0, 0.55, 0.81, 1.51, 2.0, 2.5 kGy). The results of this study have shown that there were significant increases in the shelf-life of the treated cheese. Furthermore, they indicated that the electron-beam irradiation at the dose of 2.0 kGy may inhibit the growth of spoilage microorganisms such as coliforms and

Pseudomonas spp. without affecting the sensorial characteristics of the product (Huo et al., 2013).

The efficacy of X-ray irradiation to improve the safety and quality of raw tuna fillets was also investigated by Mahmoud et al., (2016). In this study, raw tuna fillet samples were inoculated (10^8 10^9 CFU/ml) of a three-strain mixture of *Salmonella enteric* and treated to X-ray treatments (0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6 kGy). The results of this work indicated that more than a 6 log reduction of *Salmonella* population was achieved with 0.6 kGy. Furthermore, treatment with X-ray significantly reduced the initial microflora on raw tuna fillets and the levels were significantly lower than the control samples throughout the shelf-life storage at 5, 10 or 25 °C for 25, 15 or 5 days, respectively.

The effects of ionizing radiation are not limited to inactivation of the microorganisms in the products, they are also able to delay the maturation and/or germination of fruits and vegetables by disrupting endogenous enzymatic mechanism responsible for the maturation and/or germination (Kader, 1986; Singh and Pal, 2009).

1.2.4.4 Advantages and limitation of Ionizing radiation

This technology has caused and continues to cause still controversy about its applicability. It has been subjected to inspections so deepened to assess the consequences, regarding toxicology, nutritional and organoleptic aspects. The Joint FAO / IAEA / WHO Expert Committee on the Wholesomeness of Irradiated Food in 1988 and the Joint FAO / IAEA / WHO Study Group in 1997 agreed that the available toxicological studies do not indicate any adverse effects produced by the processed food consumption, both with low that with high doses of ionizing radiation (Liberty et al., 2013).

Among the advantages, certainly the most important relate to the greater safety of food and the reduction of deterioration, with a consequent reduction in food loss. In fact, this treatment could also bring benefits to consumers in terms of availability and quantity, storage life, convenience, and improved hygiene of the food. This technology do not induce radioactivity in foods or packaging materials. Furthermore, ionizing radiation did not affect the sensory and nutritional characteristics of the products processed. In fact there are no significant changes in the amino acid, fatty acid and vitamins of treated food, if they

are applied following the good practice of irradiation never exceed the recommended dose of 10 kGy (Liberty et al., 2013). Moreover, it is not secondary that this technology requires a low consumption of energy and, therefore, a reduced environmental impact compared to other storage methods (Farkas, 2006).

A big advantage of irradiated food is that it is a cold process: the food is still essentially "raw", because it hasn't undergone any thermal process. Radiation treatment causes practically no temperature rise in the product. Irradiation can be applied through packaging materials including those, which cannot withstand heat. This means also that radiation treatment can be performed also after packaging thus avoiding re-contamination or re-infestation of the product.

Among the disadvantages there is the selective loss of vitamins, which is still comparable to that seen in other forms of storage (Dionísio et al., 2009). This technology at doses within the practical limits is not able to completely inactivate viruses, enzymes and microbial toxins present in the food at the time of treatment (Liberty et al., 2013).

1.2.5 Plasma treatment

1.2.5.1 Definition and general concept

Plasma sometimes referred to as the fourth state of matter, is generated by using electricity and a carrier gas, such as air, oxygen, nitrogen, or helium. It is produced when a gas is excited by electricity or electromagnetic waves in the radio frequency or microwave range (Tendero et al., 2006). The term "plasma" refers to a partially or wholly ionized gas composed essentially of photons, ions and free electrons as well as atoms in their fundamental or excited states possessing a net neutral charge (Laroussi et al., 2012).

Two classes of plasma, namely thermal and non-thermal (or cold) plasma can be distinguished on the basis of conditions in which they are generated. The thermal plasma is generated at higher pressures and required high power. In contrast, cold plasma (near ambient temperature 30-60 °C) is obtained at atmospheric o reduced pressure (vacuum) and required less power (Fridman and Kennedy 2004). The common electrical discharges used to generate cold plasma are the corona discharge, the dielectric barrier discharge (DBD), the microwave discharge (MD), and a special arrangement called plasma jet. Plasma generation at atmospheric pressure is of interest, both technically and industrially for the food industries because this does not require extreme conditions (Laroussi et al., 2012).

Corona discharge

Corona discharge is usually generated on sharp electrodes, such as tips, pit points, or thin wires, with imposed high voltage. The electric field of high intensities is formed close to such points and the active region of corona and plasma generation occurs (Scholtz et al., 2015).

Dielectric barrier discharge (DBD)

Dielectric barrier discharge (DBD) is alternating current generated discharge burning between two electrodes separated by a solid dielectric material (glass, plastic). While the corona discharge active region appears only close to the point electrode, limited up to units of mm, the DBD electrodes are typically metal plates and therefore the plasma area is more

big. This approach shows significant potential for the treatment of various foods, with the food package in contact with high voltage electrodes (Klockow and Keener 2009).

Microwave discharges (MD)

Microwave discharges (MD) are generated by electromagnetic waves with frequencies exceeding hundreds of MHz. Due to the necessity for a microwave generating apparatus and the need for shielding, this type, in general, was often used in the basic research of cold plasma interactions with biomaterials or for medical use (Isbary et al., 2013).

Plasma jet

Another configuration is the plasma pen or jet, in which a stream of gases can be directed at the object to be treated. Generally, the active region of used discharge is blown by flowing auxiliary gas, which pulls the particles outside the electrode area in propagating ionization waves and forms a stream of active particles burning as a small jet. Kim et al. (2010) developed a cold plasma jet operating at 20 kHz Alternating Current (AC) under atmospheric pressure. The most versatile of the plasma systems is the freedom to select a gas or gas mixture.

1.2.5.2 Cold Plasma treatment on microorganisms: mechanisms of action.

The microbial inactivation effect of plasma treatment can be attributed to the formation of a number of antimicrobial products in the air: UV, radiation, ozone, charged particles and "supercharged" oxygen. All of these products work together to kill pathogens (bacterial, viral). Microorganisms in plasma are exposed to an intense bombardment by the radical most likely provoking surface lesions that the living cell cannot repair sufficiently faster. This may partially explain the observations wherein cells are in many cases destroyed very quickly. This process is termed "etching" (Pelletier 1992).

Besides, the production of UV photons of different wavelengths has been proposed to be involved in dimerizing the thymine bases of DNA. Roth et al., (2010) revealed that UV-C radiation is the most effective inactivation agent in the plasma.

The role of the charged particles in the bacterial inactivation process was investigated by Lu et al. (2009). While Perni et al. 2007 interplayed bacterial inactivation kinetics with

optical emission spectroscopy, and identified oxygen atoms as major contributor in plasma inactivation with minor contributions from UV photons, OH radicals, singlet oxygen and nitric oxide.

In another work Ehlbeck et al. (2011) evaluated the efficacy of cold plasma treatment on different microorganisms. Approximately 20 types of microorganisms (Gram-positive and Gram-negative aerobic and anaerobic bacteria, viruses and yeasts) are compared in this work to ensure process efficiency. Depending on the conditions cold plasma treatment can inactivate the microorganisms and significantly decrease (up to 5-7 magnitude) the number of CFU in suspensions or on surfaces within 10-1800 s.

It is necessary to highlight that the process parameters play an important role in the microbial inactivation. The concentrations in which the plasma agents occur in plasma depend greatly on the device set-up (reactor geometry), operating conditions (gas pressure, type, flow, frequency and power of plasma excitation) and gas composition. By varying the process parameters involved in plasma generation, a multitude of mechanisms can be actuated which may act individually or synergistically. The details of interaction of the different plasma agents with the different components of bacterial cells are currently very limited (Moisan et al., 2001; Fernández and Thompson 2012; Scholtz et al., 2015).

1.2.5.3 Cold plasma treatment: the application

The combination of highly energetic plasma species with a non-thermal treatment makes this technology suited for decontamination of food (Yu et al., 2006). The potential applications for the food industry including the dry disinfection of food surfaces (like meat, poultry, fish and freshly harvested horticultural produce), granular and particulate foods (dried milk, herbs and spices) and sprouted seeds. This technology has also been successfully applied for the surface sterilization of packaging materials and also their functional modification for imparting desired properties (Deilmann et al., 2008; Güleç et al., 2006)

One of the first applications of atmospheric plasma on contaminated apples, cantaloupe and lettuce was published by Critzer et al. (2007). The food matrices were inoculated by a mixture of *E. coli* O157:H7, *L. monocytogenes* and *Salmonella* (7 log CFU per sample). *E.*

coli O157:H7 populations were reduced of 1 and 2 log cycles after 30 s and 1 min exposures, respectively. *Salmonella* populations were reduced after 1 min by 2 log cycles and the longer exposures (3 and 5 min) yielded 3 and 5 log cycles of reductions.

Selcuk et al. (2008) successfully decontaminated the seeds of tomato, wheat (Triticum durum), bean, chickpea, soybean, barley, oat, rye, lentil (Lens culinaris) and corn, contaminated with *Aspergillus parasiticus* 798 and *Penicillium* spp. to less than 1% of initial count depending on treatment times.

Song et al. (2009) investigated the influence of food matrix. They inoculated a mixture of three *L. monocytogenes* strains into sliced cheese and ham and exposed them to plasma for 60, 90 and 120 s. Microbial log reduction appeared to be directly proportional to input power and exposure time. The results confirmed that inactivation of *L. monocytogenes* depends strongly on the type and structure of investigated of food.

Bermúdez-Aguirre et al. (2013) exposed to cold plasma, inoculated lettuce, carrots and tomatoes with a pathogenic strain of *E. coli* and assessed the microbiological quality after treatment. The degree of inactivation was dependent on the concentration of inoculum, it was easy to inactivate low bacterial concentrations.

Low temperature gas plasma sterilization allows fast and safe sterilization of packaging materials such as plastic bottles, lids and films without affecting the properties of the material or leaving any residues (Deilmann et al., 2008). This is also evident from the work of Muranyi et al. (2007) where Polyethylene terephthalate (PET) was used as the treatment medium with reported inactivation of several micro-organisms.

However, the type of materials and appropriate treatment conditions have to be considered for achieving satisfactory inactivation levels (Yun et al., 2010).
Plasma deposition of heat sensitive materials such as vitamins, antioxidants and antimicrobials into the packaging material may be sought as potential alternative in the antimicrobial and active packaging (Fernandez-Gutierrez et al., 2010).

1.2.5.4 Advantages and limitation of cold plasma technology

The cold plasma technology is an emerging disinfection method that offers a non-thermal approach for reducing the microbial populations on the raw or fresh products surface and packaging materials. Atmospheric pressure plasma system, resulting in reduced cost, increased treatment speed, and industrial applicability (Yoon and Ryu 2007; Yun et al., 2010). The ability to generate non-thermal plasma discharges at atmospheric pressure makes the decontamination process easier and less expensive (Kim et al. 2011). It offers the advantage of being chemical and water-free because the antimicrobial products are formed in air. This non-thermal processing technique is environmentally friendly and sustainable. When used on food surfaces, cold plasma does not affect the vitamin content, structure, appearance, or color of the food, and leaves no residue (Scholtz et al., 2015).

The treatment of bulky and irregularly shaped food limits the cold plasma efficacy for food sterilization, restricted volume or size of the product has to be considered. Microbial inactivation occurs usually on the treated surface, because the penetration of reactive particles into the deeper layers of food is limited (Scholtz et al., 2015).

Though the technology is promising, more research is required before it can be industrially applied.

2. Objective

Extending the shelf life of Fiordilatte cheese is an important issue to the dairy industry due to the high interest in extending the distribution of the traditional product beyond the local market. The need for enhancing microbial food safety and quality, without compromising the nutritional and sensory characteristics of foods has created an interest in non-thermal innovative processes for food preservation. UV light, Pulsed light, combined Steam-Ultrasound and X-ray are proposed as promising alternative technologies to be applied to dairy sector because are effective to control the microbial quality of this fresh cheese. The effects of these technologies on two principal spoilage microorganisms (*Pseudomonas* spp. and Enterobacteriaceae) of fresh cheese and its sensory quality are still unknown.

The main objective of this Thesis was to investigate the suitability of UV light, Pulsed light, combined Steam-Ultrasound, X-ray and Plasma jet as non-thermal preservation strategies for sanitization of cheese, in order to obtain a product safe and sensory acceptable for a longer time.

To achieve this main objective the work was divided in these following parts:

1. UV-C light treatment
   - Determination of parameters relevant for the effectiveness of UV-C light treatment for the inactivation of spoilage microorganisms of cheese and evaluation of its effectiveness on inoculated samples (preliminary tests);
   - Evaluation of UV-C light effects on Fiordilatte shelf life;
2. Pulsed light (PL) treatment
   - Determination of parameters relevant for the effectiveness of Pulsed light treatment for the inactivation of spoilage microorganisms of cheese and evaluation of the effectiveness on inoculated samples (preliminary tests);
   - Evaluation of Pulsed light effects on Fiordilatte shelf life;

3. High intensity light pulse (HILP) treatment

- Determination of parameters relevant for the effectiveness of HILP processing for the inactivation of spoilage microorganisms and evaluation of the effectiveness of this treatment on inoculated cheese (preliminary tests);

- Evaluation of the effects of HILP on native spoilage bacteria during storage period;

4. Combined Steam-Ultrasound treatment.

- Determination of parameters relevant for the effectiveness of combined Steam-Ultrasound treatment for the inactivation of spoilage microorganisms and evaluation of the effectiveness of this treatment on inoculated Fiordilatte cheese (preliminary tests);

- Evaluation of the effects of combined steam-ultrasound treatment on native spoilage bacteria during storage period;

5. X-rays treatment.

- Determination of parameters relevant for the effectiveness of X-rays treatment for the inactivation of spoilage microorganisms (preliminary tests);

- Evaluation of X-rays effects on Fiordilatte shelf life;

6. Plasma jet treatment.

- Determination of parameters relevant for the effectiveness of plasma treatment for the inactivation of spoilage microorganisms (preliminary tests);

3. Material and methods

3.1 Fiordilatte cheese

The study were done with Fiordilatte cheese, since the work has been carried out with different technologies in five different locations, the products are purchased in each location to ensure freshness. After reception, the Fiordilatte cheeses were stored a 4 °C before the treatments.

In Italy the experiments with UV-C light and Pulsed Light were performed using a thermostated cell, at 8 °C equipped with 4 UV-C lamps and a Pulsed Light mobile decontamination unit (Claranor, Rouaine, France), respectively, located at the Department of Food Science, University of Udine (Italy). The Fiordilatte cheese ("bocconcini Vallelata" 30 g) was purchased in a local market (Udine, Italy). Afterwards, the experiments with the X-rays were performed at the Institute Zooprofilattico of Puglia and Basilicata (IZS) in Foggia. The Fiordilatte cheese (100 g) was purchased in a local cheese factory "Granarolo spa" (Bologna, Italia). Finally the treatments with Plasma Jet were performed at the Department of Chemistry, University of Bari Aldo Moro (Bari, Italy) and the samples ("bocconcni Vallelata", 30 g) were purchased in a local market (Foggia, Italy).

In Denmark the experiments with combined Steam-Ultrasound were performed by a pilot equipment located at the SonoSteam laboratories, FORCE Technology (Brøndby, Denmark). The Fiordilatte cheeses (125 g) were purchased in a Danish local market (Copenhagen, Denmark).

In Ireland the experiments with High Intensity Light Pulses were performed by using the Steri-Pulse XL 3000 Pulsed Light Sterilization System (Xenon Corporation, MA, USA), located at Department of Agriculture and Food Science, University College of Dublin

(Dublin, Ireland). The Fiordilatte cheeses (30g) were purchased in a local market (Dublin, Ireland).

3.2 Investigated spoilage microbial groups

For each preservation strategy reported below, preliminary *in-vitro* tests were conducted. *Pseudomonas fluorescens* (DSM 50090), *Pseudomonas putida* (DSM 591) and Enterobacteriaceae were used as inoculum on cheese surface. The *Pseudomonas fluorescens* and *Pseudomonas putida* strains were grown overnight at 25 °C in Plate Count Broth (PCB Oxoid) and then grown on Plate Count Agar (PCA Oxoid) for 24 h at 25 °C. The Enterobacteriaceae were previously isolated from chicken skin, were grown overnight at 37 °C in Violet Red Bile Glucose Agar (VRBGA, Oxoid) and then grown on Plate Count Agar (PCA, Oxoid) for 24 h at 25 °C. Following, the colonies obtained from the relevant cultural media were used as initial microbial suspension and were subjected to serial dilutions in Maximum Recovery Diluent (MRD), depending on the microbial concentration to be reached.

Before UV-C light and Pulsed light treatments the samples (bocconcini "Vallelata" 30 g) were inoculated by dipping cheese for 5 min in the above described microbial inoculum (microbial cocktail of *P. fluorescens* and *P. putida*) to reach a final contamination of 10^4-10^5 cfu/g. Fiordilatte cheese samples were then stored at 9 °C overnight before each preservation treatments. While, in the case of the Plasma Jet the same concentration (10^4-10^5 cfu/mL) of the microbial suspension (cocktail of *P. fluorescens* and *P. putida*) was subjected to the plasma treatment.

As regards the combined Steam-Ultrasound treatments, the samples (Fiordilatte cheese 125 g) were inoculated by mean of 300 µL of microbial suspension (*P. fluorescens* and Enterobacteriaceae), directly spread on the surface of samples. Fiordilatte cheese was then stored under refrigerated condition for 2 h before the treatment, in order to reach the contamination levels of interest. In particular, the efficacy of this treatment on inoculated Fiordilatte cheese with a microbial concentration of 10^4-10^5 cfu/g, was tested. Subsequently, the efficacy of this treatment was also assessed at different microbial

concentrations, specifically 10^2-10^3 cfu/g for Enterobacteriaceae and 10^3-10^4 cfu/g for *P. fluorescens*.

As regard the High Intensity Light Pulses treatment, at first both *P. fluorescens* and Enterobacteriaceae suspensions (10^8 cfu/mL) were exposed to HILP. Afterwards, the dairy products (Fiordilatte cheese 30 g) were inoculated by means of 150 µL of microbial suspension (*P. fluorescens* and Enterobacteriaceae) directly spread on the sample surface. Fiordilatte cheese was then stored under refrigerated condition for 2 h before the HILP treatment in order to allow microorganisms to attach to the cheese surface (10^4-10^5 cfu/g).

3.3 Treatments applied to Fiordilatte cheese

The experiments were carried out to determine the suitability of these innovative techniques for the decontamination of Fiordilatte cheese. The main purpose is to preserve the quality as long as possible, thereby minimizing any damage that traditional treatments can cause, without affecting the sensory quality of these products.

For each preservation strategy, several devices have been proposed, that are described out below.

3.3.1 UV-C light treatment

UV-C light treatments were carried out at 8 °C into a thermostated cell (Climacell 222, Sylvania, SLI lighting, Raunheim, Germany) equipped with 4 UV-C lamps with emission in the range 200-280 nm (15W, OF, OSRAM, GmbH HNS, Munich Germany, maximum emission: 253.7 nm). The lamps were positioned at 2 cm from the surface of the samples. A schematic representation of the equipment is reported in figure (Figure 8). The irradiance on the cheese surface was 20 W/m^2 and samples were treated for increasing time up to 750 s. Irradiance (W/m^2) was multiplied by treatment time (s) to obtain the total fluence (J/m^2) of the treatment.
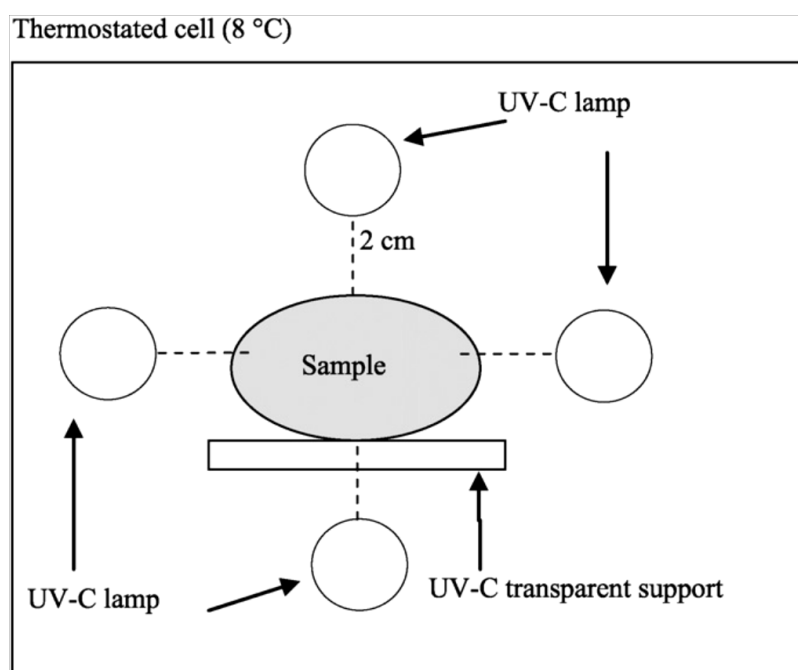
Figure 8 *Schematic representation of UV-C light treatment of Fiordilatte cheese.*

Before each test, the termostated cell was cleaned and sanitized with commercial liquid soap and alcool 70°. The experiments were run in a room at room temperature (20 °C) and the temperature in the treatment chamber was never been above 8 °C.

The study was divided in two steps:

1. In the first, inoculated Fiordilatte samples were treated by UV-C light at 20 W/m^2 for 5, 30, 60, 150, 300, 450 and 750 s corresponding to specific fluence values: Ctrl, A (0.1 kJ/m^2), B (0.6 kJ/m^2), C (1.2 kJ/m^2), D (3.0 kJ/m^2), E (6.0 kJ/m^2), F (9.0 kJ/m^2) and G (15 kJ/m^2). After treatments, samples were immediately analyzed for microbiological counts.

   UV-C light trasmittance was also evaluated. UV-C penetration depth in Fiordilatte cheese was determined photometrically using a luminometer (HD-2102.2 Delta Ohm, Padova, Italy) equipped with UV-C light probe (LP471 UVC, Padova, Italy) as described by Manzocco, et al., (2011). Sections of Fiordilatte cheese tissues of increasing thickness up to 1 mm were manually cut by a sharp blade. Sample thickness was measured by a digital caliper (ABS Digimatic,Mitutoyo Corporation, Kawasaki, Japan). Fiordilatte cheese sections were positioned on the luminometer sensor and exposed to 20W/m^2 UV-C light. The irradiance of the light transmitted through Fiordilatte tissues was measured. The ratio between transmitted light (I_0) and incident light (I) was measured and fitted by the Beere-Lambert law:

   $$I/I_0 = e^{-\alpha x} \qquad \text{(Eq. 5)}$$

   where x is the Fiordilatte thickness and α is an experimental parameter. The penetration depth (δ) was then calculated as the reciprocal of the experimental parameter α.

2. In the second step, the inactivation of naturally cheese spoilage microorganisms was determined and a shelf-life test was carried out. Fiordilatte cheeses were treated by UV-C light at the selected fluence values on the basis of the preliminary tests: Ctrl (no treatment), H (0.1 kJ/m^2), I (0.6 kJ/m^2), L (1.2 kJ/m^2) and M (6.0 kJ/m^2).

Untreated and UV-C treated Fiordilatte cheese were packaged in 14 x 14 cm polyethylene pouches (2 samples per pouch) with 50 mL brine (0.6 % NaCl solution) and stored at 9 ± 1 °C. Microbial count, pH and sensory evaluation were monitored during storage.

3.3.2 Pulsed light treatment

PL treatments were performed by using a pulsed light mobile decontamination unit (Claranor, Rouaine, France) equipped with 4 xenon lamps with emission in the range 200-1000 nm, located in the Department of Food Science, University of Udine (Italy). Lamps were positioned at each side of a quartz plaque held in the centre of the chamber. Samples were placed on the quartz plaque at 2 cm distance from the lamps. A representation of the equipment was reported in figure 9.
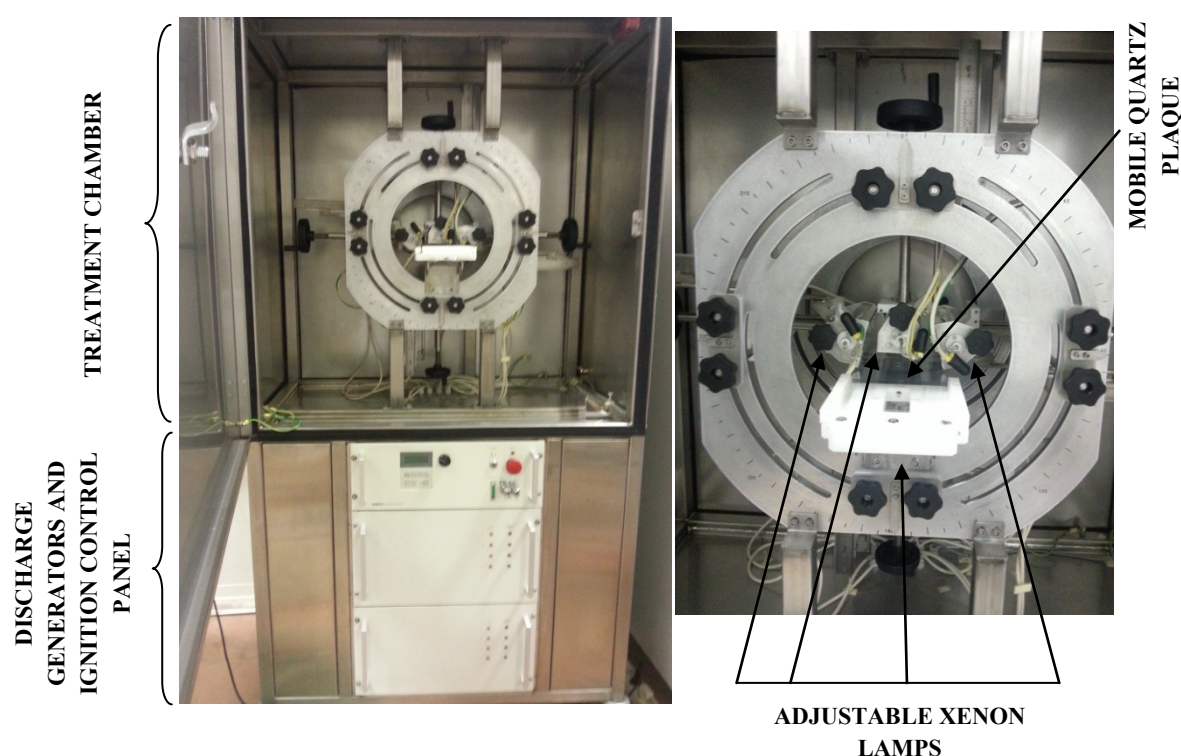
Figure 9 *Claranor, pulsed light mobile decontamination device.*

Before each test, the mobile quartz plaque was cleaned and sanitized with commercial alcool 70°. The experiments were run in a room at room temperature (20 °C).

The study was divided in different steps:

1. Preliminary tests on Fiordilatte cheese have been performed to evaluate the efficacy of the treatment. Operating at an electric potential of 1000, 2000, 2500 or 3000 V, according to the manufacturer's indications, the fluence delivered to the sample was equal to 0.20, 0.60, 0.95 or 1.6 J/cm^2/pulse, respectively.

2. Afterwards, experiments were carried out with Fiordilatte cheese samples inoculated with the above-mentioned *Pseudomonas* microbial inoculum (*P. fluorescens* and *P. putida* 10^5-10^{6}cfu/g). In agreement with preliminary tests, electric potential of 2000 and 3000 V, with 1, 3, 10 light pulses have been chosen. The treated samples were mentioned as follows: Control (Fiordilatte cheese 30g inoculated) untreated, PL.1 (3000 V 1 p), PL.2 (3000 V 3 p), PL.3 (3000V 10 p), PL.4 (2000V 1 p), PL.5 (2000V 3 p), PL.6 (2000V 10 p). After treatments, samples were immediately analyzed for microbiological counts.

3. PL trasmittance was also evaluated. PL penetration depth in Fiordilatte cheese was determined photometrically using a luminometer (HD-2102.2 Delta Ohm, Padova, Italy) equipped with Visible light probe (LP471 PHOT, Delta Ohm, Padova, Italy) as described by Manzocco, et al., (2011). Sections of Fiordilatte cheese tissues of increasing thickness were manually cut by a sharp blade. Sample thickness was measured by a digital caliper (ABS Digimatic, Mitutoyo Corporation, Kawasaki, Japan). Fiordilatte cheese sections were positioned on the luminometer sensor and exposed to 600 lux visible light. The irradiance of the light transmitted through Fiordilatte tissues was measured. The ratio between transmitted light (I$_0$) and incident light (I)was measured and fitted by the Beere-Lambert law:

$$I/I_0 = e^{-\alpha x} \qquad \text{(Eq. 5)}$$

where **x** is the Fiordilatte thickness and **α** is an experimental parameter. The penetration depth (**δ**) was then calculated as the reciprocal of the experimental parameter **α**.

4. Subsequently, two shelf-life tests were carried out to evaluate the efficacy of the treatment on native spoilage bacteria, during storage. The samples were treated at 3000 V (1, 3, 10 pulses) and 2000 V (1, 3, 10 pulses). The samples were mentioned as follows: Control (untreated Fiordilatte 30 g), 1.6 J/cm^2 (3000 V 1 pulse), 4.8 J/cm^2 (3000 V 3 pulses), 16 J/cm^2 (3000 V 10 pulses).

   Control (untreated Fiordilatte 30 g), 0.6 J/cm^2 (2000 V 1 pulse), 1.8 J/cm^2 (2000 V 3 pulses), 6.0 J/cm^2 (2000 V 10 pulses).

   Untreated and PL treated Fiordilatte cheese were packaged in 14 x 14 cm polyethylene pouches (2 samples per pouch) with 50 mL brine (0.6 % NaCl solution) and stored at 9 ± 1 °C. Microbial count, pH and sensory evaluation were monitored during storage.

3.3.3 High Intensity Light Pulses (HILP) treatment

HILP treatments were performed by using the Steri-Pulse XL3000 Pulsed Light Sterilization System (Xenon Corporation, MA, USA). It is a complete benchtop sterilization system with controller and separate sterilization chamber. It consists of a stainless steel sterilization chamber (Figure 10) with a lamp housing mounted on top and a control module (Model No. RC 747, Xenon). The controller provides power to the lamp housing as well as complete operator control of the sterilization process. Safety interlocks are provided to protect the user from exposure from the pulsed lamp when the chamber door is open. The xenon lamp emits high intensity light in the wavelength between 200 nm and 1100 nm, with a maximum emission in the UV range. All samples were placed at a distance of 2.5 cm from the light source and exposed to HILP (frequency 3 Hz; pulse width of 360 µs; fluence 1.17 J/cm^2/pulse).
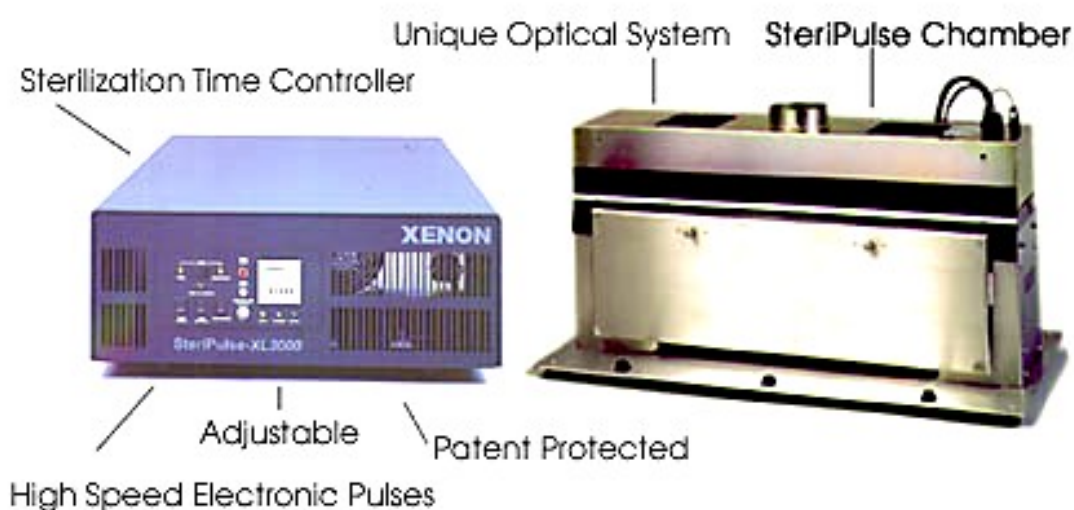
Figure 10 *Steri-Pulse XL3000 Pulsed Light Sterilization System (Xenon Corporation).*

Before each test, the stainless steel chamber was cleaned and sanitized with commercial alcool 70°. The experiments were run in a room at room temperature (circa 20 °C).

Specifically, the study was organized in three phases:

1. In the first one, the susceptibility of microorganisms (10^8 cfu/mL) to the treatment in a transparent liquid matrix (Maximum Recovery Diluent, MRD) was evaluated (*in-vitro* tests). 15 mL of both *P. fluorescens* and Enterobacteriaceae suspensions were placed in sterile petri dishes of 90 mm diameter (Sterilin Limited, United Kingdom), resulting in a liquid depth of 2 mm. These samples were then exposed to HILP at increasing treatment times from 0.4 to 8 s, in order to receive total energy doses ranging from 1.17 to 28 J/cm^2, respectively.

2. Subsequently, the surface decontamination efficacy of HILP on inoculated Fiordilatte samples (i.e. 10^4-10^5 cfu/g) was investigated. In this case treatment times from 1 to 8 s were chosen for treatments. The samples were mentioned as follows:
   Ctrl (untreated Fiordilatte); A (3.5 J/cm^2), B (7.0 J/cm^2), C (10.5 J/cm^2), D (14.0 J/cm^2), E (17.5 J/cm^2), F (21.0 J/cm^2), G (24.5 J/cm^2), H (28.0 J/cm^2).

3. On the third phase, the effect of HILP on normal samples was evaluated. Treatment times of 2 and 4 s were chosen for treatments with corresponding energy intensities of 7.0 (sample B) and 14.0 (sample D) J/cm^2, respectively. After the treatment, untreated and HILP treated samples were packaged in commercial plastic trays (PP, thickness 69 mm) with 50 mL brine (0.6% NaCl solution) and stored at 9 ± 1 °C for 2 weeks. Periodically, the microbial quality and pH were monitored.

3.3.4 Combined Steam-Ultrasound treatment

Combined steam-ultrasound treatments were performed by a steam-ultrasound pilot-scale equipment located at the SonoSteam® laboratories (FORCE Technology, Brøndby, Denmark). The device is consists in a conveyor belt, that are able to transport Fiordilatte cheese in a stainless still treatment chamber (Figure 11). It is equipped with 6 nozzles (3 on the top and 3 on the bottom), these particular designed nozzles (EPO; 02 722 020.12113) (EP1381399-B1) are able to simultaneously produce steam at 90-95 °C and ultrasound at 20-40 kHz. A control module allows to set and to check the various process parameters (treatment time, ventilation %, temperature on Top and Bottom of the treatment chamber), in such way to allow the operator to have complete control of the sterilization process. For this study 2 different nozzles were used (A= 2.7-3.4 bar; H= 2.4-3.1 bar) that differ only for the emitted steam pressure (bar).
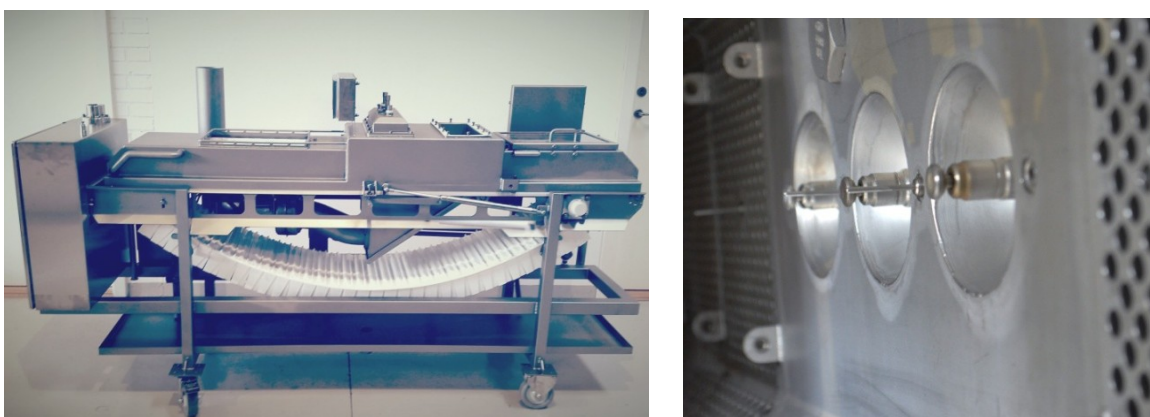
Figure 11 *SonoSteam® equipment picture.*

All Fiordilatte cheese samples were treated using the combined steam-ultrasound for increasing time up to 6 s. In particular, the work was divided in two phases:

1. For the *in-vitro* tests, the inoculated samples (10^4-10^5 cfu/g) were treated, using the nozzle **A** (2.7 bar), for 0.5, 1, 2, 4 and 6 s compared to untreated control. Afterwards, the same treatments were performed using the nozzles **H** (2.4 bar). Subsequently, the efficacy of this treatment was also assessed at different microbial concentrations, specifically 10^2-10^3 cfu/g for Enterobacteriaceae and 10^3-10^4 cfu/g

for *P. fluorescens*, using the nozzles **A**. After treatments, samples were immediately analyzed for microbial growth.

2. On the basis of the preliminary *in-vitro* experiments, in the second phase, the samples were treated by SonoSteam system using the following selected conditions: nozzles **A** (pressure 2.7 bar) and treatment times 1 and 6 seconds. Samples were named as follows:

Control (untreated Fiordilatte cheese), B (Fiordilatte treated for 1 s) and E (Fiordilatte treated for 6 s). After the treatment, untreated and combined steam-ultrasound treated samples were packaged in commercial plastic trays (PP, thickness 69 mm) with 100 mL brine (0.6% NaCl solution) and stored at 9 ± 1 °C for 2 weeks. Periodically, the microbial quality and pH were monitored.

3.3.5 X-rays treatment

The X-rays treatments were performed by using the RS-2400 (Rad Source, USA) installed at the Institute Zooprofilattico of Puglia and Basilicata (IZS) in Foggia. RS-2400 is a cabinet X-ray irradiator with exposure chamber and the electronic control housed outside of the system (Figure 12). Inside the exposure chamber there is the X-ray tube and a system for holding the sample canisters. It has the option of rotating the canisters around the X-ray source to ensure uniform exposure to all samples.

Figure 12 RS 2400 irradiator (Rad Source)

In this study a potential application of this technology on Fiordilatte cheese to prolong its shelf-life was investigated. All packaged cheeses (Granarolo 100 g) were subjected to X-ray irradiation at doses of 0.5, 2 and 3 kGy. Untreated control and treated samples were then stored at 9 ± 1 °C. The experimental analyses were conducted in two replicates. Microbiological count, pH and sensory quality were monitored.

3.3.6 Plasma jet treatment

The Plasma Jet treatment, located at the Department of Chemistry, University of Bari Aldo Moro was adopted. The scheme of plasma jet is shown in Figure 13. A stainless steel capillary is inserted into a glass tube (90 mm jet immersed or 70 mm jet on the surface). A slight gap is formed between the capillary and the tube in this way. On the outside of the glass tube is present a strip that it serves as a grounded electrode. The capillary is connected to a power supply. The plasma jet is mounted in a small chamber allowing the control of the ambient atmosphere. Helium or a mixture Helium + Oxygen is guided in the plasma source through the annular space between the glass tube and the capillary and serves as a plasma forming gas.
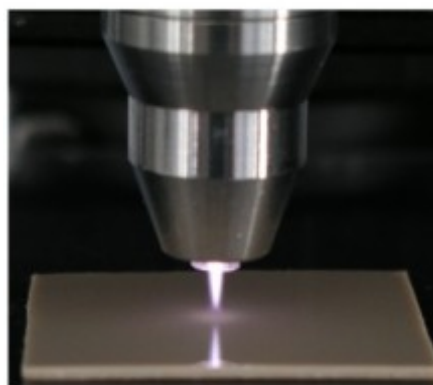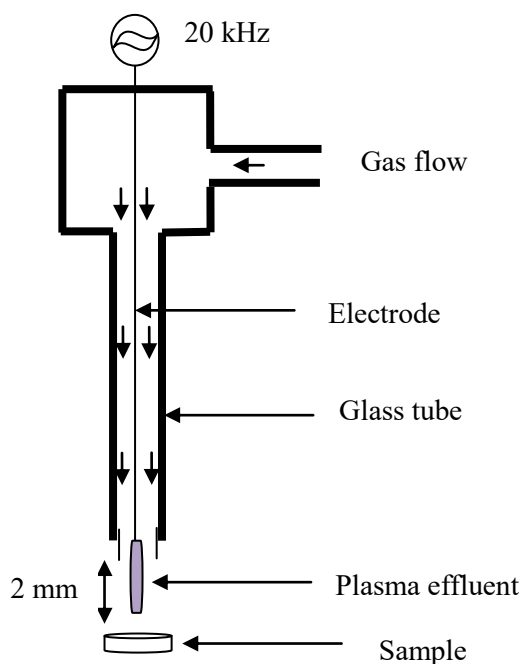
Figure 13 Scheme of plasma jet.

The study was divided in two steps:

1. In the first one, the microbial inoculum (*P. fluorescens* and *P. putida* 10^4-10^5 cfu/mL) were subjected to the plasma treatment using Helium (He) and mixture Helium + Oxygen with different Oxygen concentrations (0.15 %; 0.25 %; 0.40 %) for treatments with plasma jet immersed, and the same gas carries at various

concentrations (0.15 %; 0.25 %; 0.40 %; 0.55 %) for treatments with plasma jet on the surface. The duration of each treatment was 15 minutes. The treated samples were mentioned as follows: PLASMA.1 (He), PLASMA.2 (He/O_2% 0.15 %), PLASMA.3 (He/O_2 0.25 %), PLASMA.4 (He/O_2 0.40 %), plasma jet immersed. PLASMA.5 (He), PLASMA.6 (He/O_2% 0.15 %), PLASMA.7 (He/O_2 0.25 %), PLASMA.8 (He/O_2 0.40 %), PLASMA.9 (He/O_2 0.55 %), plasma jet on the surface. A control sample (CNT), represented by untreated Saline solution + inoculum 10^4-10^5 cfu/mL (10ml) was also considered.

2. On the second step, the Fiordilatte cheeses (30g) in 50ml of brine were subjected to plasma treatment using as process gas mixture Helium + Oxygen (0.40%; 0.55%). The treatments were performed using the plasma jet on the surface and the plasma jet immersed with a treatment time of 60 minutes. The treated samples were mentioned as follows: CNT (Fiordilatte cheese 30g + 50ml of brine) untreated, CAMP.1 (He/O_2 0.40 %), CAMP.2 (He/O_2 0.55 %) plasma jet on the surface, CAMP.3 (He/O_2 0.40 %), CAMP.4 (He/O_2 0.55 %) plasma jet immersed.

The long treatment times, the plasma application mode and the ineffectiveness were the problems related to the potential utilization of this non-thermal technology. Therefore, the investigations with this technology were aborted and only few results were produced, discussed in a small paragraph.

3.4 Analysis carried out on Fiordilatte cheese

3.4.1 Microbiological analysis

Microbiological analysis of food, is aimed basically to ensure that the foods respect specified microbiological criteria. Microbiological analysis may provide information relating to the microbiological safety of food, the respect of the rules of Good Manufacturing and Distribution of food and the effect of storage on food quality.

The microbiological analysis is intended to determine the number of microorganisms or, more precisely, the number of Colony Forming Units (CFU) per ml or g in a given sample. The number of microorganisms present in a given food sample, in most cases, is so high that it cannot be counted accurately if not after having been subjected to serial dilutions. Typically, in microbiology, a food sample is serially diluted in the ratio 1:10 (sample: diluent).

Once prepared the serial decimal dilutions of samples it is necessary to transfer these in the appropriate nutrient medium. The serial decimal dilutions are generally of 1 ml or 0.1 ml in function of the inoculum method used. The aim is to inoculate the plate, with an ever smaller number of microorganisms, so that to have, after incubation in optimal conditions, well isolated colonies in number between 30 and 300, so as to be easily counted.

There are two main techniques of plating:

a) Technique "Pour Plate"

With this technique, empty sterile Petri plates are inoculated (1 mL of the serial dilutions) and then covered with the suitable agar medium maintained at 45 ° C. The growth of the colonies occurs both on the surface and in depth. The colonies incorporated in the matrix of the substrate, in some cases, may be difficult to be numbered, especially at low dilutions where it may be confused with the particles of the food sample.

b) Technique "Spread plate" (Surface distribution of the inoculum on a solid substrate).

With this technique it is prepared before the Petri plates with the suitable agar medium. Only after the medium has solidified, the plates are inoculated with the sample serial dilutions (0.1 mL). The growth of the colonies occurs on the surface, facilitating their numbering.

The substantial difference of this technique compared to that of inoculum for inclusion, resides in the fact that it makes use of plates containing the substrate already ready, which are inoculated with volumes of 0.1 ml of each dilution. You must take this into account in the reading phase, multiplying the N° of Colony Forming Units/g or ml counted by a factor of 10.

After to have inoculated the plates with one of the techniques previously described, they should be incubated in thermostats at temperatures and optimal growth times, that depend on the type of microbial population that you want to research.

Relatively to these studies, for the inoculated Fiordilatte cheese samples, the microbiological analysis were aimed to count concentration of *Pseudomonas* spp. and Enterobacteriaceae. Counting was carried out on each Fiordilatte sample by classic plate counting technique. Ten grams of Fiordilatte was aseptically removed from each sample, diluted with 90 mL of maximum recovery diluent (MRD) or saline solution (NaCl) in a stomacher bag and homogenized with a Stomacher LAB Blender 400. Subsequently, decimal serial dilutions of homogenates were made using the same diluent and the dilutions were plated on appropriate media in Petri plates. *Pseudomonas* spp. were determined on Pseudomonas Agar Base (PAB, Oxoid) properly modified by adding Pseudomonas C-F-C SR103 (Oxoid) selective supplement after autoclaving at 121 °C for 15 min; plates were incubated for 48 h at 25 °C. While for enumeration of Enterobacteriaceae Violet Red Bile Glucose Agar (VRBGA, Oxoid) was used, plates was incubated for 24 h at 37 °C.

Shelf-life tests were performed to evaluate the effect of these novel non-thermal technologies on microbial growth during storage at 9±1 °C, taking untreated Fiordilatte cheese as a reference. All samples were treated using the optimal doses (specific for each

applied technology) identified in the preliminary studies. Immediately after the treatments, the samples (treated and untreated) were aseptically transferred in sterile pouches (14 × 14 cm polyethylene) or in commercial plastic trays (PP, thickness 69 mm) with brine and stored at 9±1 °C. During the shelf-life, two microbial groups were taken into account *Pseudomonas* spp. and Enterobacteriaceae. When these microbial groups at the end of the shelf-life exceed the value 10^6 cfu/g and 10^5 cfu/g, respectively, the product can be rejected, from a microbiological point of view.

In the microbiological analysis, during the shelf-life test, appropriate media were used to enumerate specific spoilage groups. Specifically, Plate Count Agar (Oxoid) and Violet Red Bile Glucose Agar (VRBGA, Oxoid) were used for enumeration of mesophilic and Enterobacteriaceae; plates were incubated for 48 h at 30 °C and 24 h at 37 °C, respectively. Pseudomonas Agar Base (PAB, Oxoid), was used for enumeration of *Pseudomonas* spp. concentration. Lactic acid bacteria and lactococci were plated on MRS Agar (Oxoid) and M17 Agar (Oxoid) respectively, supplemented with cycloheximide (0.1 g/L Sigma) and Lactose (Oxoid) solution 10% only for lactococci. The Petri plates were incubated at 37 °C for 48 h. All analyzes were performed in duplicate on two different batches. Colony forming units were counted and expressed as log cfu/g (Isohanni & Lyhs, 2009).

The experimental data were fitted by means of a modified version of the Gompertz equation (Eq. 6), re-parameterized by Corbo, Del Nobile, & Sinigaglia (2006):

$$\log\big(N(t)\big) = \log(N_{max}) - A$$

$$\cdot \exp\left\{-exp\left\{\left[(\mu_{\max} \cdot 2.71) \cdot \frac{\lambda - MAL}{A}\right] + 1\right\}\right\}$$

$$+ A \cdot \exp\left\{-exp\left\{\left[(\mu_{\max} \cdot 2.71) \cdot \frac{\lambda - t}{A}\right] + 1\right\}\right\}$$

(Eq. 6)

where **N(t)** is the viable cell concentration at time **t**, **A** is related to the difference between the decimal logarithm of maximum bacterial growth attained at the stationary phase and decimal logarithm of the initial value of cell concentration, $\mu_{\max}$ is the maximal specific

growth rate, λ is the lag time, $\mathbf{N_{max}}$ is the microbial threshold value, $\mathbf{MAL}$ is the microbiological acceptability limit (i.e., the time at which $\mathbf{N(t)}$ is equal to $\mathbf{N_{max}}$), and $\mathbf{t}$ is the storage time. The value of $\mathbf{N_{max}}$ was set to 10^6 CFU/g for *Pseudomonas* spp. (MAL^{Pseudomonas}) and 10^5 CFU/g for Enterobacteriaceae (MAL^{Enterobacteriaceae}), respectively (DPR 54/97). This allowed quantifying the microbial acceptability limit (MAL), defined as the storage time at which the viable cell concentration reaches its threshold value.

3.4.2 pH determination

The pH values on each sample were determined by direct reading whit pH-metre (Crison, Barcelona, Spain), after appropriate calibration. The pH was evaluated on homogenates of cheese and on the brine. Each value was the average of measures recorded on sample from two different batches.

3.4.3 Sensory analysis

A quantitative descriptive analysis was used for the comparison of samples (UNI 10957:2003, Sensory analysis-Method to define the sensory profile of foods and beverages). During the shelf-life test, seven trained judges of the laboratory evaluated the quality of Fiordilatte cheese.

In accordance with the standard UNI 10957:2003, seven testers of the laboratory were selected on the basis of international standards ISO 8586-1:1993 and ISO 8586-2:1994. The selection was made considering various aspects: interest and motivation, eating habits (consumption of Fiordilatte), ability to communicate sensations and time available for analysis sessions. During the sensorial analysis sessions, the Fiordilatte samples were simultaneously presented to each panelist without brine, randomly coded. The panelists were asked to evaluate cheese odor, color, texture and overall quality every day for the entire period of observation, by using a scale from 0 to 7 (Corradini & Innocente, 2002), as the main parameters of fresh "pasta filata" cheese (Chen, Wolle, & Sommer, 2009). A value of 4 indicated the limit at which the overall quality of product was considered unacceptable.

To judge the overall quality of cheese the following product characteristics were taken into account: color white porcelain, smooth surface, elastic release of buttermilk after cutting, lack of holes and typical milk smell. The analysis of the texture was performed by touching the surface of the products with fingers and evaluating the degree of surface fraying with movements from top to bottom of the surface. At the same time samples were subjected to a small pressure, crushing them between fingers in order to evaluate the degree of the elasticity and the ability to release the typical milk drop without relaxing. The sensorial analysis was performed on different days in correspondence of the microbiological analysis.

In order to quantitatively determine the efficacy of non-thermal technology (specifically UV-C light, Pulsed light and X-ray treatments), the sensory acceptability limit (SAL), intended as the number of days within Fiordilatte remained acceptable from the sensory point of view, was also calculated. To this aim, the modified version of the Gompertz equation (Eq. 7), re-parameterized by Corbo et al. (2006) was fitted to sensory data:

$$SA(t) = SA_{min} + A^{SA}$$

$$\cdot \exp\left\{-exp\left\{\left[(\mu_{max}^{SA} \cdot 2.71) \cdot \frac{\lambda^{SA} - SAL}{A^{SA}}\right] + 1\right\}\right\}$$

$$+ A^{SA} \cdot \exp\left\{-\exp\left\{\left[(\mu_{max}^{SA} \cdot 2.71) \cdot \frac{\lambda^{SA} - t}{A^{SA}}\right] + 1\right\}\right\}$$

(Eq. 7)

where **SA(t)** is the sensory attribute at time t, $\mathbf{A^{SA}}$ is related to the difference between the sensory attribute attained at the stationary phase and the initial value of sensory attribute, $\boldsymbol{\mu_{max}^{SA}}$ is the maximal rate at which **SA(t)** decreases, $\boldsymbol{\lambda^{SA}}$ is the lag time, $\mathbf{SA_{min}}$ is the sensory attribute threshold value, **SAL** is the sensory acceptability limit (i.e., the time at which SA(t) is equal to SA_{min}), and **t** is the storage time. The value of $\mathbf{SA_{min}}$ is equal to 4, as the threshold for cheese acceptability, according to the scientific literature (Del Nobile et al., 2009; Gammariello et al., 2009).

3.5 Statistical analysis and shelf life calculation

Analyses were performed in duplicate on at least two different samples. Results are reported as mean value ± SD. The statistical analysis was performed by one-way ANOVA test. A Duncan's multiple range test, with the option of homogeneous groups (p < 0.05), was used to determine significance among differences. To this aim, STATISTICA v. 7.1 for Windows (StatSoft Inc., Tulsa, OK, USA) was used. In this work the shelf life was calculated as the lowest value between MAL^{Pseudomonas}, MAL^{Enterobacteriaceae} and SAL, as also reported in other works dealing with shelf-life of Fiordilatte cheese (Gammariello et al., 2009, 2011).

4.   Results and discussions

4.1 UV-C light treatment

4.1.1 Efficacy of UV-C light on inoculated samples

To verify the inactivation efficacy of UV-C light, its effect was initially studied on *Pseudomonas* spp. inoculated on Fiordilatte cheese. These microorganisms were chosen as representative of spoilage microorganisms, potentially leading to product quality depletion and shelf-life reduction.

Table 3 shows the counts of inoculated *Pseudomonas* spp. as a function of the different UV-C light fluence. As expected, *Pseudomonas* spp. was near the inoculated concentration in the control. Exposure to UV-C light for increasing time caused an overall decrease of microbial population by 0.8 or more than 1 log cycle. The better result was observed for the sample E (6.0 kJ/m^2), even though fluences higher than 6.0 kJ/m^2 did not promote a further decrease in microbial count. No direct proportionality between treatment intensity and microbial load reduction was detected in the entire range of tested fluence, even though a function between inactivation and UV-C dose could be found with more complex mathematical models (Guerrero & Barbosa-Cánovas, 2004; Rossitto *et al.*, 2012). Similar findings were also found for UV-C light applied to pineapple sticks and could be attributed to the superficial effect of the light radiation (Manzocco *et al.*, 2015).

Table 3 Microbial counts of *Pseudomonas* spp. inoculated on Fiordilatte cheese exposed to increasing fluence UV-C light.

Samples	Fluence kJ/m^2	*Pseudomonas* Log cfu/g
Ctrl	-	4.7 ± 0.2^a
A	0.1	3.9 ± 0.2^b
B	0.6	$3.5\pm0.4^{b,c}$
C	1.2	$3.6\pm0.2^{b,c}$
D	3.0	$3.2\pm0.1^{b,c}$
E	6.0	2.6 ± 0.2^c
F	9.0	$3.2\pm0.2^{b,c}$
G	15.0	$3.1\pm0.2^{b,c}$

[a-c]Data with different letters are significantly different (P<0.05)

To estimate how deeply light can penetrate into Fiordiatte cheese, leading to microbial inactivation, the penetration depth of UV-C light was evaluated. Fig. 14 shows the ratio between transmitted (I) and incident (I$_0$) UV-C light through Fiordilatte cheese having increasing thickness. Data were fitted by the Beer-Lambert law (5). The experimental parameter α resulted to be equal to 21.57. The penetration depth (δ), computed as the reciprocal of α, was 0.05 mm. This evidence confirms that the germicidal efficacy of UV-C is limited to a very thin surface layer of the product.
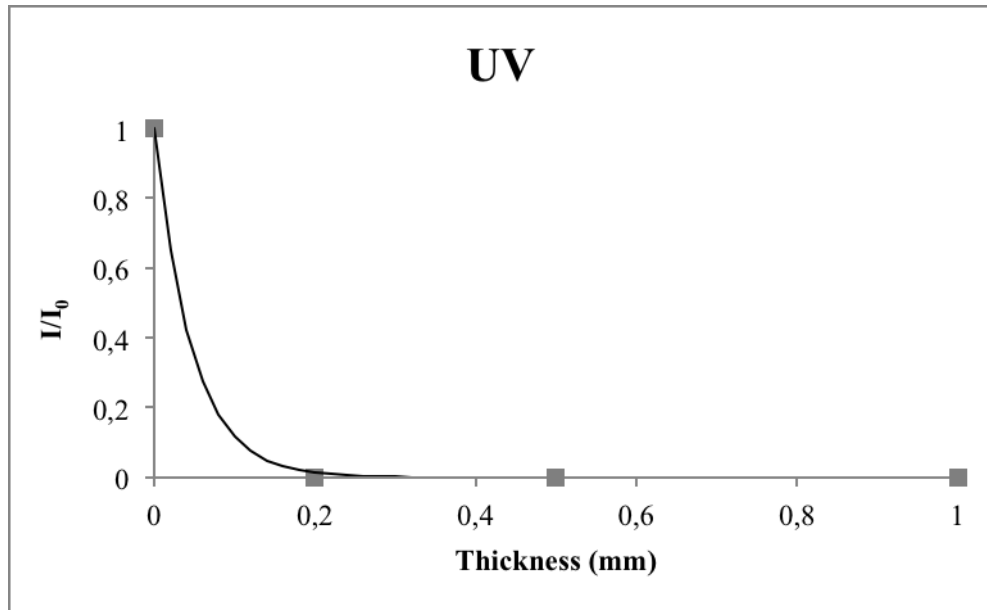
Figure 14 Ratio between transmitted (I) and incident (I_0) UV-C light in Fiordilatte cheese with increasing thickness. Symbols: experimental data. Line: estimates by Eq. (5).

4.1.2 UV-C light effects on Fiordilatte shelf life

Based on the results acquired in the preliminary test, the second part of the research was focused on the effects of selected UV-C light treatments on quality of Fiordilatte cheese. In particular, cheeses were exposed to UV-C light at 0.1, 0.6, 1.2 and 6.0 kJ/m^2 fluence. Samples were then packaged in brine and stored for increasing time for more than two weeks at 9±1 °C. The shelf-life test was thus carried out by monitoring the evolution of microbial counts and sensory quality.

The growth of spoilage microorganisms Enterobacteriaceae and *Pseudomonas* spp. was taken into particular account for determining the shelf-life. The dairy industry has a high interest to limit this microbial contamination as it could affect the odor and the texture during storage (Bevilacqua et al., 2007). Fig. 15 shows the evolution of *Pseudomonas* spp. population in Fiordilatte exposed to increasing fluence of UV-C light. A 10^6 cfu/g contamination level of *Pseudomonas* spp. was taken as the limit beyond which product alteration begins to appear (Angiolillo et al., 2014). Independently on the treatment fluence, all samples exposed to the light showed a lower initial microbial load. In addition, as the fluence was increased, a lower microbial count was progressively detected just after

the treatment. These results are in agreement with those reported by Reinemann et al. (2006), which showed that exposure to UV light was capable of reducing many bacteria in raw milk. The UV-C light efficacy on *Pseudomonas* spp. was mainly observed within the first four days of storage because after this time all the treated samples reached microbial populations similar to that recorded in the control cheese (Manzocco et al., 2015) that became unacceptable just after 2 days. These results confirm that the UV-C light treatment exerts an initial germicidal activity but it is not able to inhibit microbial growth during storage. This finding could be explained considering that UV-C light only acts on the product surface and does not significantly penetrate into the food. The initial decontamination was associated to an extension of the time needed to reach the acceptability limit by a value between 10 and 80%, depending on the treatment fluence.
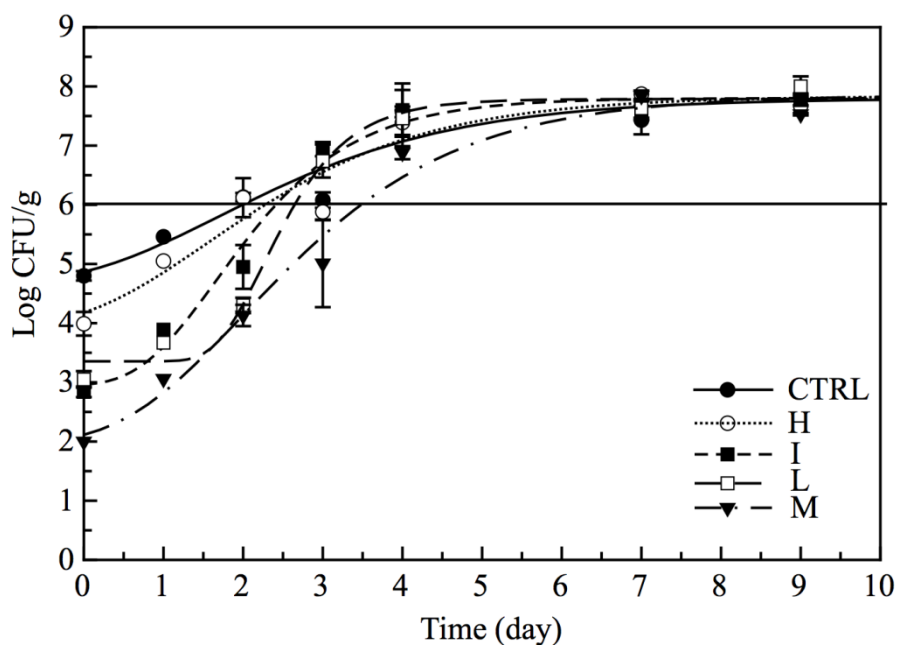
Figure 15 Evolution of *Pseudomonas* spp. population during refrigerated storage of Fiordilatte cheese exposed to increasing fluence of UV-C light. Symbols: experimental data. Lines: best fits estimates. *CTRL = untreated Fiordilatte; H = Fiordilatte cheese UV-C treated for 5 s; I = Fiordilatte cheese UV-C treated for 30 s; L = Fiordilatte cheese UV-C treated for 60 s; M = Fiordilatte cheese UV-C treated for 300 s.*

Figure 16 shows the evolution of Enterobacteriaceae population during refrigerated storage of Fiordilatte cheese treated by UV-C light. In this case, the microbial limit for unacceptability was set at 10^5 cfu/g. Data confirm that Enterobacteriaceae are more photo-sensitive than other bacteria, including *Pseudomonas* spp. (Manzocco et al., 2015; Allende et al., 2006; Manzocco et al., 2011). As can be seen in the figure, there was a gradual increase of microbial load for all samples, with different lag phases. In particular, for the control and the sample H (0.1 kJ/m^2) the increase of microbial load was observed starting from 2nd day, the acceptability limit (10^5 cfu/g) was overlapped after more than 4 storage days. For the sample I (0.6 kJ/m^2) the microbial load started to increase from the 3th day and overlapped the threshold after more than 5 days. A different trend was observed for the samples L (1.2 kJ/m^2) and M (6.0 kJ/m^2) because these samples did never exceed the microbiological limit during the entire observation period.
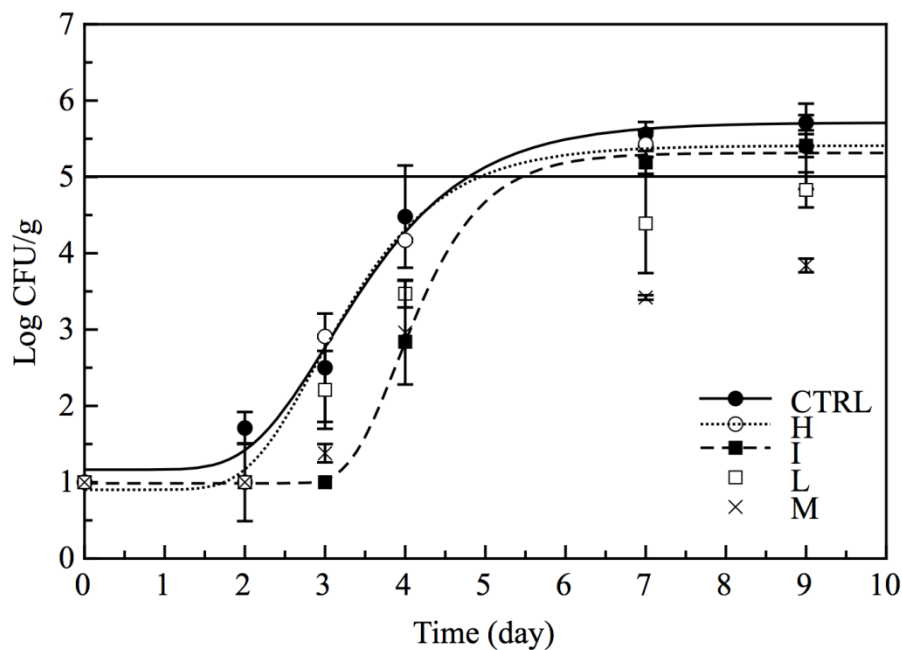
Figure 16 Evolution of Enterobacteriaceae population during refrigerated storage of Fiordilatte cheese exposed to increasing fluence of UV-C light. Symbols: experimental data. Lines: best fits estimates. *CTRL = untreated Fiordilatte; H = Fiordilatte cheese UV-C treated for 5 s; I = Fiordilatte cheese UV-C treated for 30 s; L = Fiordilatte cheese UV-C treated for 60 s; M = Fiordilatte cheese UV-C treated for 300 s.*

As regards typical dairy microorganisms, no inhibition was observed. In fact, the evolution of lactic acid bacteria and lactococci was similar for treated and untreated samples (data not shown). This result is supported by the fact that the pH of all samples remained almost constant during the entire observation period in the range 6.0-6.1 in cheese and 5.8-6.0 in brine, thus suggesting that UV-C light did not change the microbial activity that generally induces changes in pH (Orlowska et al., 2013).

The effects of the UV-C light treatment on the sensory properties of Fiordilatte cheese are reported in Fig. 17. The sensory acceptability limit (SAL) was set in correspondence to a score value equal to 4 on a scale 0-7. As expected, during cheese storage, the overall quality score progressively decreased in all the samples with similar trends. In particular, the control and the sample M reached the sensory threshold after *circa* 8 days, the sample L after *circa* 9 days, the samples H and I were very near to the sensory limit at the end of the observation period, even if they never reached it. The most striking feature of treated samples was observed just after the treatment for the cheese exposed to the highest UV-C light fluence (M, 6.0 kJ/m^2) which showed a lower overall quality than all the other samples, due to the perception of a slight anomalous odor described by the panelists as "burned". As also reported above, even though odor affected the general sensory score this sample remained acceptable for more than one week. Reinemann et al. (2006) also observed cooked, burned and rancid off-flavors in UV-C treated milk at dose of 1.5 kJ/L. The formation of off-odors could be mainly attributed to the photo-reactivity of proteins. The latter are known to be major targets for photoreactions due to the abundance of amino acid side-chains (e.g., thriptophan, tyrosine, phenylalanine, and cysteine) that can act as effective chromophores. Photo-activated proteins rapidly react resulting in the development of side-chain oxidation, backbone fragmentation, and/or formation of cross-links and aggregates (Davies, 2003). To this regard, photo-induced changes have been also reported in lactoglobulin, egg white proteins, gluten and enzymes (Fernández et al., 2012; Manzocco, et al., 2012; 2013; Panozzo et al., 2016). UV irradiation would thus favor non-enzymatic browning and S-S/SH exchange leading to the formation of sensory perceivable volatiles (Sheldon et al., 1988; Panozzo et al., 2016).

Figure 17 Evolution of the Overall quality of Fiordilatte cheese treated by UV-C light, as a function of storage times. Symbols: experimental data. Lines: best fits estimates. *CTRL = untreated Fiordilatte; H = Fiordilatte cheese UV-C treated for 5 s; I = Fiordilatte cheese UV-C treated for 30 s; L = Fiordilatte cheese UV-C treated for 60 s; M = Fiordilatte cheese UV-C treated for 300 s.*
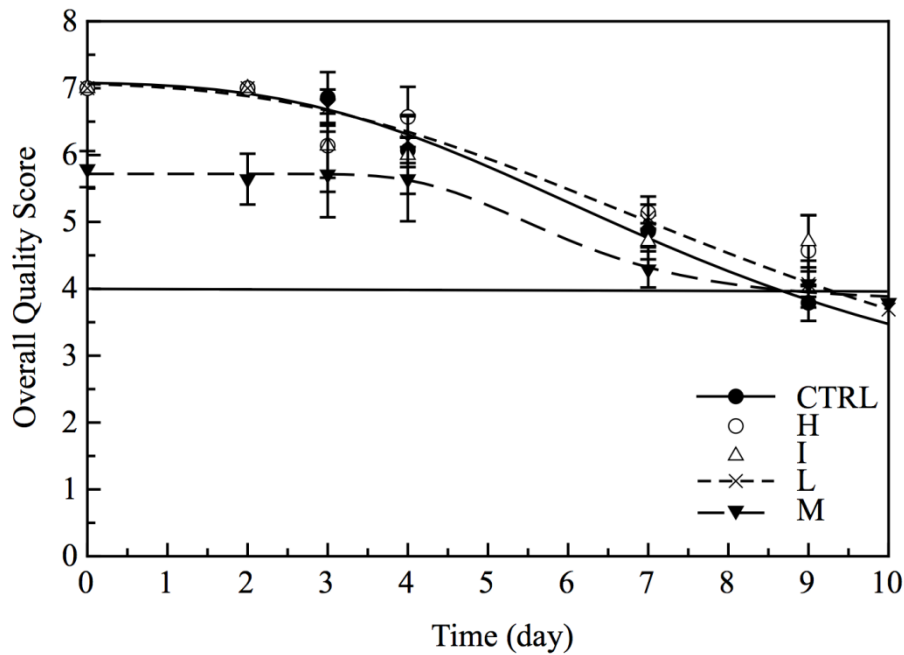
Fiordilatte shelf-life is shown in Table 4 as the lowest value among $MAL^{Pseudomonas}$, $MAL^{Enterobacteriaceae}$ and SAL (Conte et al., 2009). As can be seen, for all the samples tested in this study the microbiological quality limited the shelf-life, in particular for *Pseudomonas* spp. proliferation. Therefore, the best result was noted for the sample M (6.0 kJ/m^2) with a shelf-life of more than 3 days, compared to the untreated samples that remained acceptable for less than 2 days. The other treated samples recorded shelf-life values between them.

Table 4 Shelf-life (day) of Fiordilatte cheese as the lowest value between $MAL^{Pseudomonas}$, $MAL^{Enterobacteriaceae}$ and SAL.

Samples	Treatment time (s)	Microbial quality (day)		Sensory quality (day)	Shelf-life (day)
		$MAL^{Pseudomonas}$	$MAL^{Enterobacteriaceae}$	SAL	
Ctrl	-	1.99±0.32[a]	4.78±0.43[a]	8.59±0.43[a]	1.99±0.32[a]
H	5 s	2.27±0.31[a,b]	4.29±0.32[a]	>10	2.27±0.31[a,b]
I	30 s	2.40±0.17[a,b]	5.45±0.46[a]	>10	2.40±0.17[a,b]
L	60 s	2.64±0.12[b]	>10	9.21±0.18[a]	2.64±0.12[b]
M	300 s	3.47±0.30[c]	>10	8.50±0.54[a]	3.47±0.30[c]

[a-c]Data in column with different letters are significantly different (P<0.05).

4.2 Pulsed light treatment

4.2.1 Preliminary test on fresh cheese

The preliminary phase was aimed to test the pulsed light treatment at different intensities on Fiordilatte cheese. The combinations of processing parameters (i.e., number of pulses and electric potential) with increasing fluence, were reported in Table 5. In this first phase the preliminary test was carried out on native microflora of Fiordilatte cheese. In particular *Pseudomonas spp.* and Enterobacteriaceae were taken into account, inasmuch they are the mainly responsible for the quality deterioration of dairy products (Franciosi et al., 2011; Baruzzi et al., 2012; Martin et al., 2011).

Fiordilatte cheeses (bocconcini Vallelata 30 g) were treated with PL an electric potential of 1000, 2000, 2500 and 3000 V. According to the manufacturer's indications, the fluence delivered to the sample was equal to 0.20, 0.60, 0.95 or 1.6 J/cm^2/pulse, respectively.

From these preliminary results, as regard *Pseudomonas* spp. count, it is seen that no treatment had effect. Probably due to the high initial contamination levels (10^6 cfu/g) on Fiordilatte cheese that reduce the decontamination efficacy of this treatment. Gómez-López et al., (2005b) have observed a strong decrease in inactivation efficiency when high counts of *L. monocytogenes* were reached on the surface of an agar medium. The less efficient decontamination is due to the shadow effect, that reduced the effective radiation dose available for microbial inactivation (Lagunas-Solar et al., 2006). On the contrary for Enterobacteriaceae a better result was observed. All treatments were able to reduce the initial microbial contamination below the detection limit with *circa* 4 log cycles of microbial reduction. These results have allowed to understand that the treatment could be effective to reduce the initial contamination level, but it is markedly influenced by the initial microbial concentration.

Table 5 Effect of Pulsed Light treatment on native microflora of Fiordilatte cheese.

Electric potential (V)	Fluence per pulse (J/cm^2/pulse)	Number of pulses	Tot. fluence (J/cm^2)	*Pseudomonads* spp. Log cfu/g	Enterobacteriaceae Log cfu/g
Cnt	0	0	0	6.66±0.38[a]	4.28±0.38[a]
1000	0.20	3	0.6	6.50±0.35[a]	<DL
		5	1.0	6.40±0.21[a]	<DL
		10	2.0	6.32±0.09[a]	<DL
2000	0.60	1	0.6	6.51±0.21[a]	<DL
		3	1.8	6.64±0.02[a]	<DL
		5	3.0	6.71±0.08[a]	<DL
		10	6.0	6.56±0.05[a]	<DL
2500	0.95	1	0.95	6.68±0.04[a]	<DL
		3	2.85	6.59±0.10[a]	<DL
		5	4.75	6.73±0.11[a]	2.13±0.61[c]
		10	9.5	6.68±0.04[a]	<DL
3000	1.6	1	1.6	6.73±0.10[a]	3.49±0.26[b]
		3	4.8	6.70±0.07[a]	<DL
		5	8.0	6.39±0.37[a]	<DL
		10	16.0	6.60±0.08[a]	<DL

DL: Detection limit Enterobacteriaceae 1.00 cfu/g

[a-f] Data in column with different letters are significantly different (P<0.05).

To estimate how deeply light can penetrate into Fiordiatte cheese, leading to microbial inactivation, the penetration depth of Visible (Vis) light was evaluated. Fig. 18 shows the ratio between transmitted (I) and incident (I$_0$) Vis light through Fiordilatte cheese having increasing thickness. Data were fitted by the Beer-Lambert law (Eq. 5). The experimental parameter α resulted to be equal to 0.42. The penetration depth (δ), computed as the reciprocal of α, was 2.38 mm. This evidence confirms that the germicidal efficacy of Pulsed light is limited to a very thin surface layer of the product.
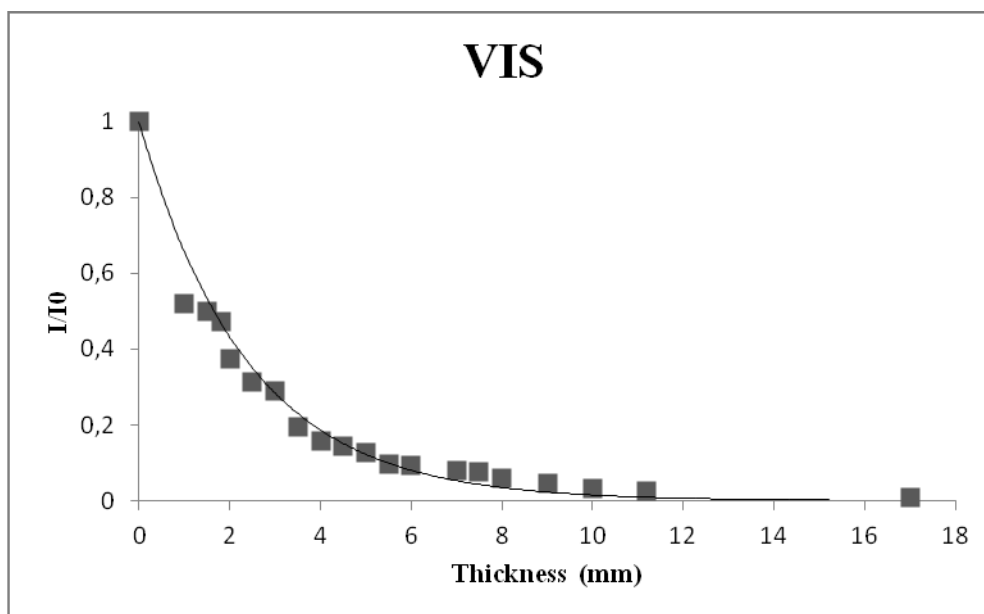
Figure 18 Ratio between transmitted (I) and incident (I_0) Vis light in Fiordilatte cheese with increasing thickness. *Symbols: experimental data. Line: estimates by Eq. (5).*

4.2.2 Efficacy of PL treatment on surface inoculated Fiordilatte cheese

Afterwards, experiments were carried out with Fiordilatte cheese samples inoculated with the above-mentioned *Pseudomonas* microbial inoculum (*P. fluorescens* and *P. putida;* 10^5-10^6cfu/g). In agreement with preliminary tests, electric potential of 2000 and 3000 V, with 1, 3, 10 light pulses have been chosen. The microbial counts for each treatment condition were determined. Figure 19 (a, b) show the behavior of *Pseudomonas* inoculated on cheese surface placed at a distance of 2 cm from the light source. The samples were exposed to PL with increasing fluence, 1.6, 4.8, 16.0 J/cm^2 for electric potential of 3000 V and 0.6, 1.8, 6.0 J/cm^2 for electric potential of 2000 V, respectively. These results showed that the microbial count for both treatment conditions decreased with the increase in light fluence (Palgan et al., 2011; Miller et al., 2012). In particular, with a treatment 3000 V and 1 pulse (PL.1), corresponding to a fluence of 1.6 J/cm^2 a slight microbial reduction of *Pseudomonas* was observed. In contrast, a PL treatment 3000 V and 3 pulses (PL.2) with a fluence of 4.8 J/cm^2, reduced the microbial loads by 1.7 log cycles for *Pseudomonas*

inoculum on cheese surface. In this case keeping fixed the electric potential and increasing the number of pulses (PL.3; 3000 V, 10 pulses) it was not observed an increase in the decontamination efficacy of the treatment. No direct proportionality between treatment intensity and microbial load reduction was detected, probably due to the more complex mechanisms that can influence the inactivation efficacy of PL treatment (Gómez-López et al., 2007; Lagunas-Solar et al., 2006; Hillegas & Demirci 2003).

On the contrary with the PL treatments performed at 2000 V with 1, 3, and 10 pulses, was observed that the increase of pulses number leads to a greater reduction of the *Pseudomonas* population (Palgan et al., 2011; Miller et al., 2012). In this case there is a direct proportionality between the treatment intensity and microbial load reduction. Significant reductions of 1.27 and 2.06 log cycle were achieved for *Pseudomonas* inoculated on Fiordilatte cheese with a fluence of 1.8 and 6.0 J/cm^2 (PL.5 and PL. 6), respectively. While in the sample PL. 4 (2000 V 1 pulse) it was observed only a slight microbial reduction of 0.79 log cycle.
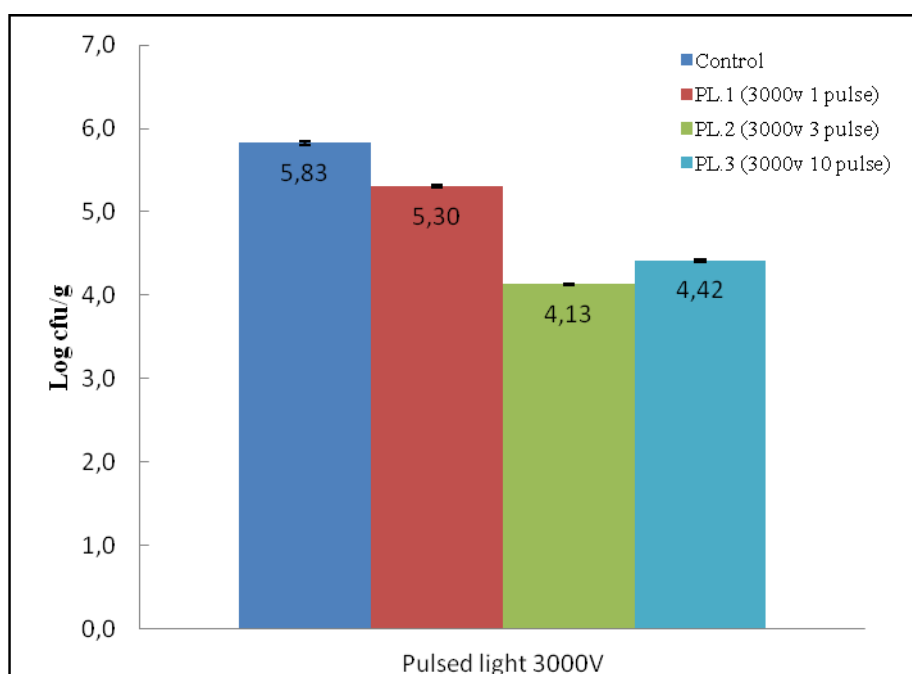
Figure 19 (a) Effect of PL treatment (3000 V; 1, 3, 10 pulses) on inoculated Fiordilatte cheese with *P. fluorescens* and *P. putida*. *Error bars represent the error with standard deviation.*
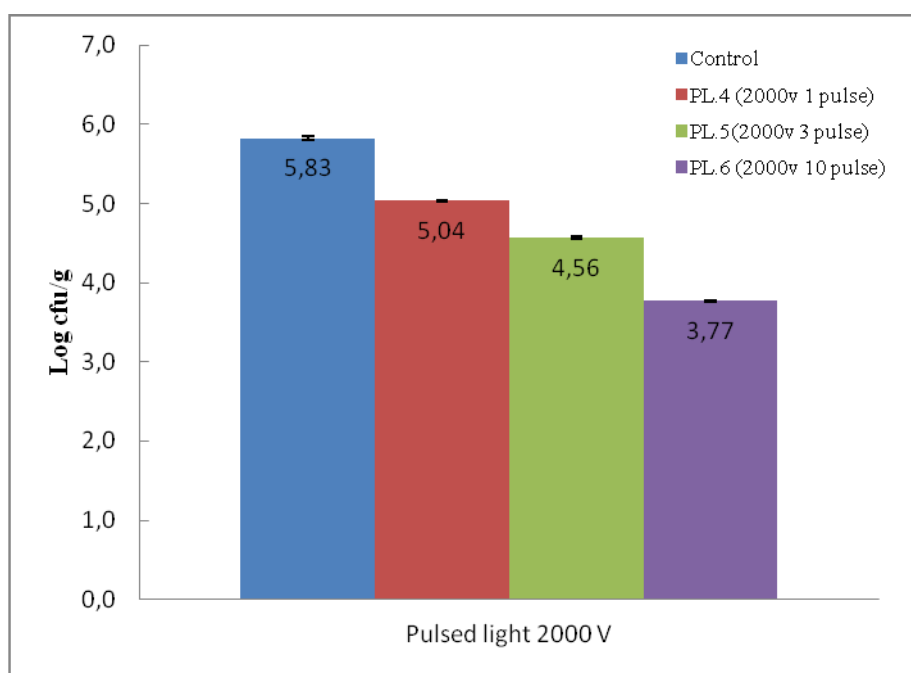
Figure 19 (b) Effect of PL treatment (2000 V; 1, 3, 10 pulses) on inoculated Fiordilatte cheese with *P. fluorescens* and *P. putida.* Error *bars represent the error with standard deviation.*

4.2.3 PL effects on Fiordilatte shelf life

Based on the results acquired in the preliminary test, the second part of the research was focused on the effects of selected PL treatments on quality of Fiordilatte cheese. In particular, cheeses were exposed to PL at fluence of 1.6, 4.8, 16.0 J/cm^2 (3000 V, 1, 3, 10 pulses) and 0.6, 1.8, 6.0 J/cm^2 (2000 V, 1, 3 and 10 pulses). Samples were then packaged in brine and stored for increasing time for more than two weeks at 9±1 °C. The shelf-life test was thus carried out by monitoring the evolution of microbial counts and sensory quality. The growth of spoilage microorganisms Enterobacteriaceae and *Pseudomonas* spp. was taken into particular account for determining the shelf-life. Figure 20 (a, b) shows the evolution of *Pseudomonas* spp. population in Fiordilatte exposed to increasing fluence of PL (3000 V and 2000 V). A 10^6 cfu/g contamination level of *Pseudomonas* spp. was taken as the limit beyond which product alteration begins to appear (Angiolillo et al., 2014). Independently on the treatment fluence, all samples exposed to the light showed a lower

initial microbial load. In addition, as the fluence was increased, a lower microbial count was progressively detected just after the treatment. The PL efficacy on *Pseudomonas* spp. was mainly observed within the first two days of storage because after this time all the treated samples reached microbial populations similar to that recorded in the control cheese that became unacceptable just after few days (*circa* 2 days). These results confirm that the PL treatment exerts an initial germicidal activity but it is not able to inhibit microbial growth during storage. This finding could be explained considering that PL only acts on the product surface and does not significantly penetrate into the food.
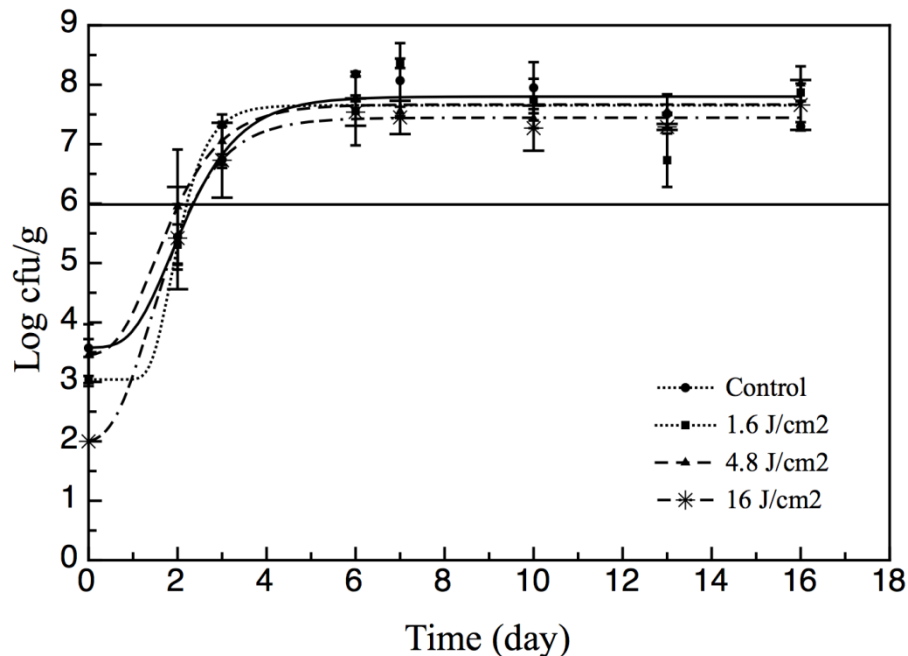
Figure 20 (a) Evolution of *Pseudomonas* spp. population in Fiordilatte exposed to increasing fluence PL (3000 V). Symbols: experimental data. Lines: best fits estimates. *Control = untreated Fiordilatte cheese; 1.6 J/cm² = Fiordilatte cheese PL treated for 3000 V with 1 pulse; 4.8 J/cm² = Fiordilatte cheese PL treated for 3000 V with 3 pulse; 16 J/cm² = Fiordilatte cheese PL treated for 3000 V with 10 pulse.*
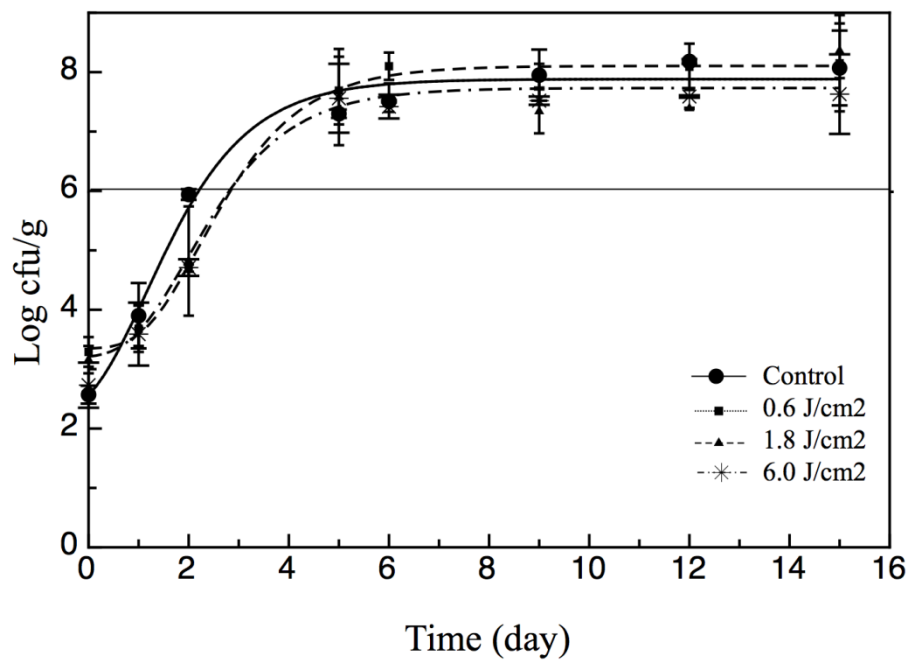
Figure 20 (b) Evolution of *Pseudomonas* spp. population in Fiordilatte exposed to increasing fluence PL (2000 V). Symbols: experimental data. Lines: best fits estimates. *Control = untreated Fiordilatte cheese; 0.6 J/cm^2 = Fiordilatte cheese PL treated for 2000 V with 1 pulse; 1.8 J/cm^2 = Fiordilatte cheese PL treated for 2000 V with 3 pulse; 6.0 J/cm^2 = Fiordilatte cheese PL treated for 2000 V with 10 pulse.*

Fig. 21 (a, b) shows the evolution of Enterobacteriaceae population during refrigerated storage of Fiordilatte cheese treated by PL (3000 V and 2000 V). In this case, the microbial acceptability limit was set at 10^5 cfu/g. Data confirm that Enterobacteriaceae are more photo-sensitive than *Pseudomonas* spp. (Innocente et al., 2014; Ignat et al., 2014; Manzocco et al., 2013). In particular, as can be seen in the figure 21 (a), there was a gradual increase of microbial load for all samples, with different lag phases. For the control the increase of microbial load was observed starting from 2nd day, the acceptability limit (10^5 cfu/g) was overlapped after more than 4 storage days. For the sample 1.6 J/cm^2 (3000 V, 1 pulse) the microbial load started to increase from the 3th day and overlapped the threshold after more than 7 days. A different trend was observed for the samples 4.8 J/cm^2 (3000 V, 3 pulse) and 16 J/cm^2 (3000 V, 10 pulse). Although there was a slight increase

116

during the first days of monitoring, these samples did never exceed the microbiological limit during the entire observation period.

Instead, for PL treatments performed at 2000 V (1, 3, and 10 pulses), as can be seen in the figure 21 (b) for all the samples the microbial load increased at the same time, starting from the first day. In particular, the Control overlaps the acceptability limit after more than 3 days, followed by sample 1.8 J/cm^2 (2000 V, 3 pulse) which reached the limit after *circa* 4 days. A different trend was observed for the samples 0.6 J/cm^2 (2000 V, 1 pulse) and 6.0 J/cm^2 (2000 V, 10 pulse), because there was a gradual increase of microbial load, with a growth near the acceptability limit around the 6th day, but the threshold was exceed after *circa* 10 and 12 days, respectivelly.

Figure 21 (a) Evolution of Enterobacteriaceae population during refrigerated storage of Fiordilatte cheese treated by PL (3000 V). Symbols: experimental data. Lines: best fits estimates. *Control = untreated Fiordilatte cheese; 1.6 J/cm^2 = Fiordilatte cheese PL treated for 3000 V with 1 pulse; 4.8 J/cm^2 = Fiordilatte cheese PL treated for 3000 V with 3 pulse; 16 J/cm^2 = Fiordilatte cheese PL treated for 3000 V with 10 pulse.*

Figure 21 (b) Evolution of Enterobacteriaceae population during refrigerated storage of Fiordilatte cheese treated by PL (2000 V). Symbols: experimental data. Lines: best fits estimates. *Control = untreated Fiordilatte cheese; 0.6 J/cm^2 = Fiordilatte cheese PL treated for 2000 V with 1 pulse; 1.8 J/cm^2 = Fiordilatte cheese PL treated for 2000 V with 3 pulse; 6.0 J/cm^2 = Fiordilatte cheese PL treated for 2000 V with 10 pulse.*

As regards typical dairy microorganisms, no inhibition was observed. In fact, the evolution of lactic acid bacteria and lactococci was similar for treated and untreated samples (data not shown). This result is supported by the fact that the pH of all samples remained almost constant during the entire observation period in the range 6.4-6.1 in cheese and in brine, thus suggesting that PL light did not change the microbial activity and the pH.

These results suggest that PL treatment had a slight effect only for the first days on Fiordilatte cheese, probably due to the shadow effect that can also occur in foods with rough or uneven surfaces, or pores. Resulting in a reduction of treatment effectiveness, because the microorganisms are able to fit into small openings and have a protective effect

(Lagunas-Solar et al., 2006). Furthermore, it was noticed that Enterobacteriaceae are more photo-sensitive than *Pseudomonas* spp. (Gómez-López et al., 2005 a; Ignat et al, 2014).

The effects of the PL treatment on the sensory properties of Fiordilatte cheese are reported in Fig. 22 (a, b). The sensory acceptability limit (SAL) was set in correspondence to a score value equal to 4 on a scale 0-7. As expected, during cheese storage, the overall quality score progressively decreased in all the samples with similar trends. In particular, for PL treatments performed at 3000 V (1, 3, and 10 pulses) figure 22 (a), the Fiordilatte cheese treated with highest fluence (4.8 and 16 J/cm^2) have reached the sensory threshold for the first, after *circa* 8 and 6 days, respectively. While a different trend was observed for the Control and the sample 1.6 J/cm^2, that they reached the sensory limit after *circa* 12 and 10 days, respectively. On the contrary, for PL treatments performed at 2000 V (1, 3, and 10 pulses) figure 22 (b), the Fiordilatte cheese treated with highest fluence (6.0 J/cm^2) and the Control have reached the sensory limit for the first, after *circa* 8 and 10 days, respectively. Instead, the samples 0.6 and 1.8 J/cm^2 overlapped the sensory limit after *circa* 11 and 12 days, respectively.

For both studies of shelf-life, the most striking feature of treated samples was observed just after the treatment for the cheeses exposed to the highest PL fluence (16 and 6.0 J/cm^2) which showed a lower overall quality than all the other samples, due to the perception of a slight anomalous odor described by the panelists as "burned". This anomalous odor was perceived immediately after the treatment, influencing the entire sensorial analysis. The formation of off-odors could be mainly attributed to the photo-reactivity of proteins. The latter are known to be major targets for photoreactions due to the abundance of amino acid side-chains that can act as effective chromophores. (Orlowska et al., 2013; Fernández et al 2012; Panozzo et al., 2016; Manzocco et al., 2013; van Aardt et al., 2005).
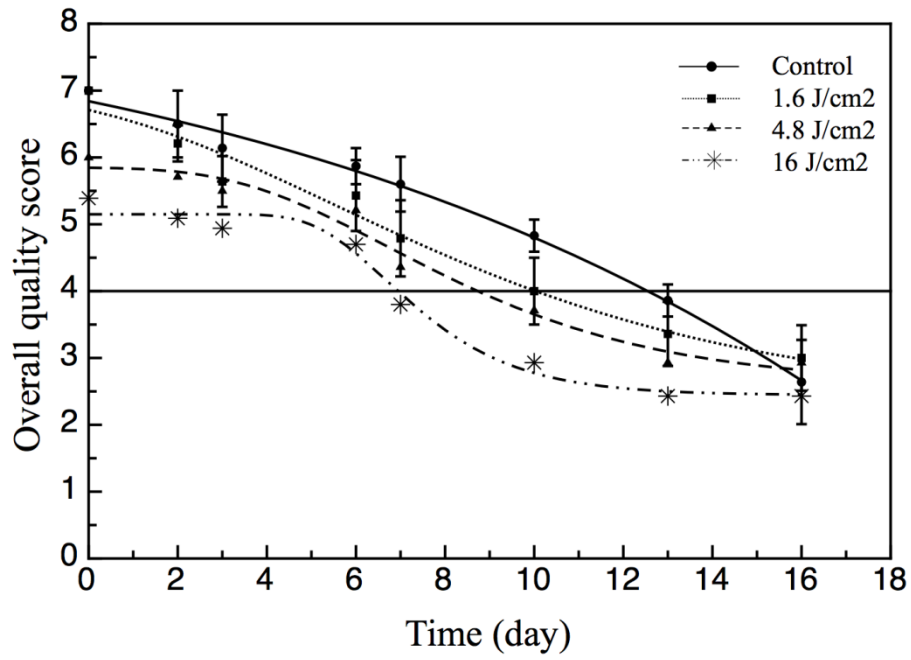
Figure 22 (a) Evolution of the Overall quality of Fiordilatte cheese treated by PL (3000 V), as a function of storage times. Symbols: experimental data. Lines: best fits estimates. *Control = untreated Fiordilatte cheese; 1.6 J/cm^2 = Fiordilatte cheese PL treated for 3000 V with 1 pulse; 4.8 J/cm^2 = Fiordilatte cheese PL treated for 3000 V with 3 pulse; 16 J/cm^2 = Fiordilatte cheese PL treated for 3000 V with 10 pulse.*
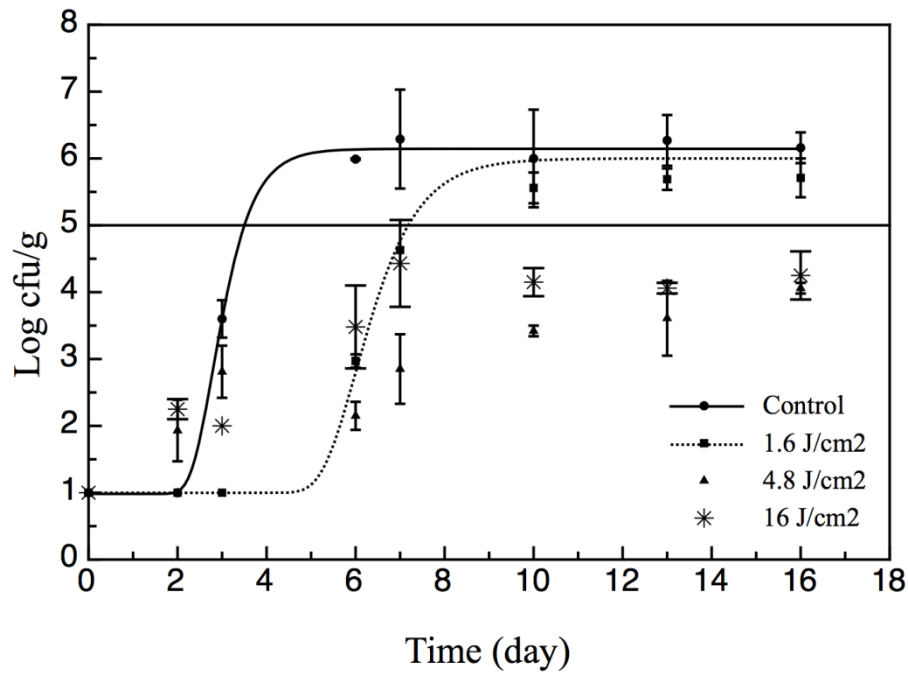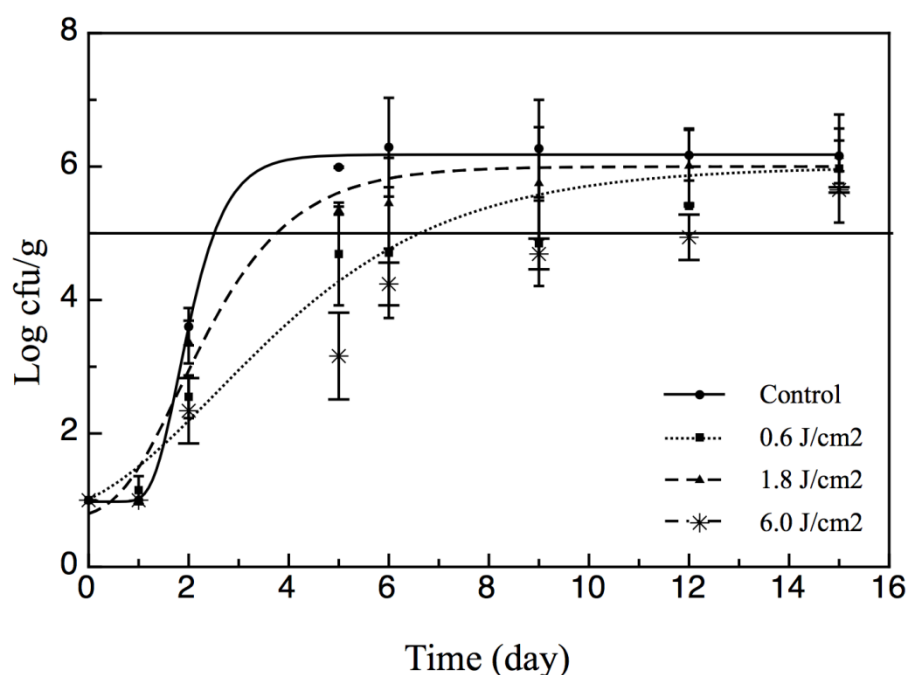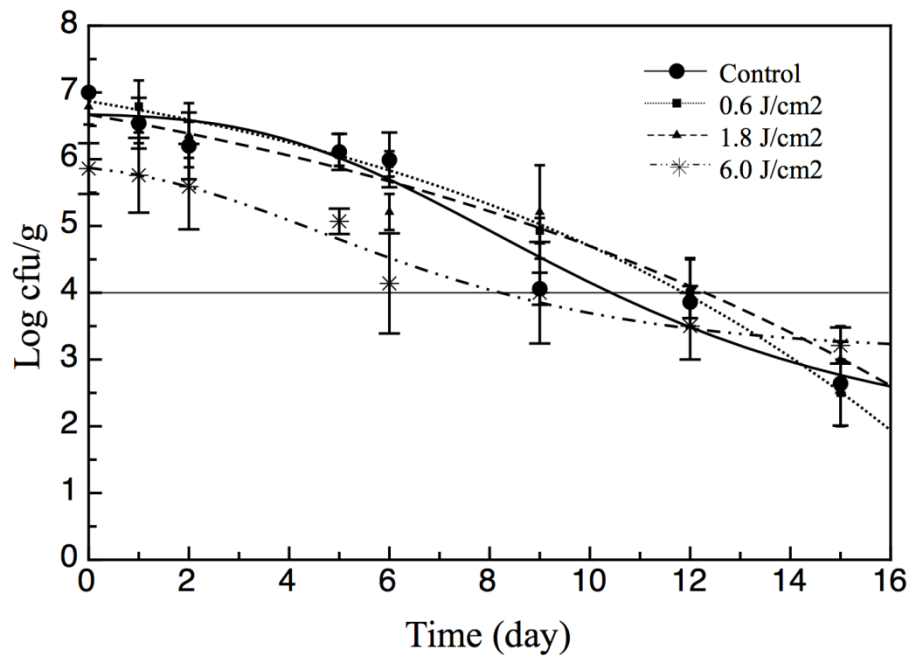
Figure 22 (b) Evolution of the Overall quality of Fiordilatte cheese treated by PL (2000 V), as a function of storage times. Symbols: experimental data. Lines: best fits estimates. *Control = untreated Fiordilatte cheese; 0.6 J/cm^2 = Fiordilatte cheese PL treated for 2000 V with 1 pulse; 1.8 J/cm^2 = Fiordilatte cheese PL treated for 2000 V with 3 pulse; 6.0 J/cm^2 = Fiordilatte cheese PL treated for 2000 V with 10 pulse.*

Fiordilatte shelf-life is shown in Table 6 (a, b) as the lowest value among MAL^{Pseudomonas}, MAL^{Enterobacteriaceae} and SAL (Conte et al., 2009). As can be seen, for all the samples tested in this study the microbiological quality limited the shelf-life, in particular for *Pseudomonas* spp. proliferation. Therefore, in this case there was not a significant prolongation of the shelf-life compared to the untreated control. A slight effect was observed for the PL treatments performed at 2000 V (1, 3, and 10 pulses) table 6 (b), all treated samples showed a shelf-life of *circa* 3 days, compared to the untreated samples that remained acceptable for *circa* 2 days. The microbial reduction observed on Fiordilatte was not much high. This is probably due to the protective effect of the cheese composition and to the fact that the cheese surface is rough and has pores or crevices where microorganisms can hide from light (Lagunas-Solar et al., 2006).

Table 6 (a) Shelf-life (day) of Fiordilatte cheese treated by PL (3000 V), as the lowest value between $MAL^{Pseudomonas}$, $MAL^{Enterobacteriaceae}$ and SAL.

Samples	Microbial quality (day)		Sensory quality (day)	Shelf-life (day)
	$MAL^{Pseudomonas}$	$MAL^{Enterobacteriaceae}$	SAL	
Control	2.35 ± 0.20^a	4.6 ± 0.20^a	12.5 ± 0.34^d	2.35 ± 0.20^a
1.6 J/cm^2	2.20 ± 0.21^a	7.4 ± 0.27^b	10.1 ± 0.61^c	2.20 ± 0.21^a
4.8 J/cm^2	2.03 ± 0.14^a	>16	8.72 ± 0.59^b	2.03 ± 0.14^a
16 J/cm^2	2.33 ± 0.10^a	>16	6.94 ± 0.27^a	2.33 ± 0.10^a

[a-d]Data in column with different letters are significantly different (P<0.05).

Table 6 (b) Shelf-life (day) of Fiordilatte cheese treated by PL (2000 V), as the lowest value between $MAL^{Pseudomonas}$, $MAL^{Enterobacteriaceae}$ and SAL.

Samples	Microbial quality (day)		Sensory quality (day)	Shelf-life (day)
	$MAL^{Pseudomonas}$	$MAL^{Enterobacteriaceae}$	SAL	
Control	2.20 ± 0.18^a	3.61 ± 0.58^a	10.4 ± 0.96^b	2.20 ± 0.18^a
0.6 J/cm^2	2.83 ± 0.12^b	10.4 ± 0.14^c	$11.8\pm0.38^{b,c}$	2.83 ± 0.12^b
1.8 J/cm^2	2.81 ± 0.44^b	4.62 ± 0.21^b	12.3 ± 0.75^c	2.81 ± 0.44^b
6.0 J/cm^2	2.85 ± 0.19^b	12.6 ± 0.18^d	8.21 ± 0.87^a	2.85 ± 0.19^b

[a-d]Data in column with different letters are significantly different (P<0.05).

4.3 High Intensity Light Pulses (HILP) treatment

4.3.1 Inactivation of spoilage bacteria in transparent liquid matrix

The preliminary experiments with HILP were performed on inoculated MRD with a microbial concentration of 10^8 cfu/mL. This study was performed to investigate the ability of different fluences to inactivate microorganisms in a transparent liquid matrix. The effectiveness of HILP was evaluated by determining the log cycles reduction of *P. fluorescens* and Enterobacteriaceae populations. The microbial counts for each treatment condition were determined. Table 7 shows their behavior in MRD, placed at a distance of 2.5 cm from the light source and exposed to HILP with increasing fluence, ranging from 1.17 to 28.0 J/cm^2. These results showed that the microbial count for both microorganisms decreased with the increase in light fluence (Palgan et al., 2011; Miller et al., 2012). In particular, with a treatment time of 0.4 second corresponding to a HILP treatment with 1 pulse and a fluence of 1.17 J/cm^2, a microbial reduction of *P. fluorescens* and Enterobacteriaceae of 2.54 and 2.12 log cycles, respectively, was observed. In contrast, a treatment time of 0.7 s corresponding to a fluence of 3.51 J/cm^2 reduced the microbial loads by 5.27 and 5.21 log cycles for *P. fluorescens* and Enterobacteriaceae, respectively. The microbial loads for both microorganisms were inactivated below the detection limit (<10 cfu/mL for *Pseudomonas fluorescens* and <1 cfu/mL for Enterobacteriaceae) after 2 seconds exposure to HILP treatment with a maximum fluence of 7.0 J/cm^2. These results showed that in a transparent liquid matrix, there was a significant microbial inactivation using this novel non-thermal technology, with just a few seconds of treatment (2 s). This finding is in agreement with what was observed in the study carried out on different liquid foods inoculated with different microbial strains (Palgan et al., 2011). Documented studies suggest that the ultraviolet (UV) portion of the spectrum is the most important for microbial inactivation (Gómez-López et al., 2007; Innocente et al., 2014). In fact the lethal effect of pulsed light on microorganisms is mostly attributed to the photochemical action of the UV portion (180-380 nm) of the spectrum emitted by the xenon flash lamp (Bintsis, Litopoulou-Tzanetaki, & Robinson, 2000; Innocente et al., 2014; Ignat et al., 2014; Oms-Oliu et al., 2010 b). This leads to a microbial DNA absorption of UV light that induces chemical modifications, with impairment of cell replication and ultimately cell death

(Gómez-López et al., 2007; Innocente et al., 2014; McDonald et al., 2002; Palgan et al., 2011; Ignat et al., 2014; Miller et al., 2012). Interestingly, the photothermal effects of IR region lead to instantaneous heat generation inside the product due to the high energy emitted during HILP treatment, without increasing excessively the temperature of the exposed sample (Miller et al., 2012; Marquenie et al., 2003; Oms-Oliu et al., 2010 b).

Table 7 Inactivation of *Pseudomonas fluorescens* (DSM 50090) and Enterobacteriaceae in Maximum Recovery Diluent (MRD) by High Intensity Light Pulses (HILP).

Treatment times (s)	Pulses	Fluence J/cm^2	*P. fluorescens* Log cfu/ml	Enterobacteriaceae Log cfu/ml
-	-	-	8.74±0.45[a]	8.63±0.15[a]
0.4	1	1.17	6.20±0.04[b]	6.51±0.09[b]
0.7	2	2.34	5.28±0.11[c]	5.76±0.04[c]
1	3	3.51	3.47±0.21[d]	3.42±0.32[d]
2	6	7.02	<DL	<DL
4	12	14.04	<DL	<DL
8	24	28.08	<DL	<DL

[a-d]Data with different letters in each column are significantly different ($P<0.05$).

DL: Detection limit for *Pseudomonas fluorescens:* 10 cfu/mL and Enterobacteriaceae*:* 1 cfu/mL in MRD, placed to 2.5 cm from the high intensity light source.

4.3.2 Surface decontamination of inoculated cheese by HILP treatment

To investigate the effectiveness of HILP treatment as surface decontamination system, Fiordilatte cheese was inoculated with *P. fluorescens* and Enterobacteriaceae, as these are the microorganisms mainly responsible for the quality deterioration during refrigerated storage (De Jonghe et al., 2011; Franciosi et al., 2011; Baruzzi et al., 2012; Martin et al., 2011). A microbial concentration of 10^4-10^5 cfu/g was used as inoculums. The inoculated samples were exposed to HILP treatment at increasing time of up to 8 seconds. Table 8 shows the microbial counts of *P. fluorescens* and Enterobacteriaceae inoculated on the product surface. There was a greater reduction for both microbial strains until 4 s exposures but then, as dose increases, the population maintained approximately constant, without significant differences in general. In particular, significant reductions of 1.07 and 1.06 log cycles were achieved for *P. fluorescens* and Enterobacteriaceae with fluences of 14.04 J/cm^2 and 7.02 J/cm^2, respectively. Similar results were obtained by Dunn et al. (1991) in a study on curds inoculated with *Pseudomonas* spp. and treated with pulsed light (fluence of 16 J/cm^2), resulting in a 1.5 log reduction of microbial population after the treatment. These observations are also in agreement with another study where reductions up to 1.69 log cycles for different microbial strains on chicken exposed to HILP have been achieved (Haughton et al., 2011). Other treatment times did not result in a significant reduction compared to untreated control, the reduction was less than 1 log cycle, this could be attributed to a non effective access of the radiation due to the irregular cheese surface (Guerrero-Beltrán & Barbosa-Cánovas, 2004). Of the two organisms studied Enterobacteriaceae appear slightly more sensitive to HILP treatment. In fact, at the same treatment conditions (fluence of 3.5 J/cm^2) there were a 0.99 and a 0.55 log cycle reduction in Enterobacteriaceae and *P. fluorescens* samples respectively. The microbial reduction observed on Fiordilatte was not as high as that detected in the preliminary study conducted on liquid matrix (MRD). This is probably due to the protective effect of the cheese composition vs. a liquid buffer and to the fact that the cheese surface is rough and has pores or crevices where microorganisms will hide from light (Palgan et al., 2011; Innocente et al., 2014; Choudhary & Bandla, 2012; Guerrero-Beltrán & Barbosa-Cánovas, 2004).

Table 8 Microbial counts of *Pseudomonas fluorescens* (DSM 50090) and Enterobacteriaceae inoculated on Fiordilatte cheese exposed to increasing fluence of High Intensity Light Pulses.

Samples	Treatment times (s)	Fluence J/cm^2	*P. fluorescens* Log cfu/g	Enterobacteriaceae Log cfu/g
Control	-	-	4.82±0.03[a]	4.65±0.14[a]
A	1	3.51	4.27±0.05[b]	3.66±0.06[c]
B	2	7.02	3.91±0.29[b,c]	3.59±0.02[c]
C	3	10.53	3.83±0.08[c]	3.76±0.02[b,c]
D	4	14.04	3.75±0.02[c]	3.88±0.04[b]
E	5	17.55	3.97±0.02[b,c]	3.77±0.10[b,c]
F	6	21.06	3.99±0.17[b,c]	3.75±0.11[b,c]
G	7	24.57	3.92±0.44[b,c]	3.71±0.01[b,c]
H	8	28.08	3.99±0.13[b,c]	3.74±0.14[b,c]

[a-c] Data with different letters in each column are significantly different (P<0.05)

4.3.3 HILP effect on Fiordilatte cheese

On the basis of the work on liquid and inoculated cheese samples, in the second phase of this study the effectiveness of HILP treatments on the native flora of Fiordilatte cheese was investigated without any preliminary contamination. Treatment parameters chosen were 2 s (sample B, figure 23) and 4 s (sample D, figure 23), corresponding to fluences 7.02 and 14.04 J/cm^2, respectively. For Enterobacteriaceae, no proliferation has been found in both control and treated samples. The absence of this microbial group allows us to hypothesize that good hygienic conditions were adopted during cheese making process (Gammariello et al., 2011). Figure23 shows the evolution of *Pseudomonas* spp. during refrigerated storage in Fiordilatte exposed to two different HILP treatments compared to untreated control. The horizontal line at 10^6 cfu/g indicates the microbial limit above which changes to the quality parameters generally begin to appear (Angiolillo et al., 2014; Mastromatteo et al., 2015 b; Cantoni et al., 2003). As it can be seen, the initial microbial load for all samples was below the detection limit ($<10^2$ cfu/g). After a few days of storage (4 days), an increase of *Pseudomonas* spp. population was observed in the control samples, and the microbiological limit was exceeded after *circa* 5 days. By contrast, for the HILP treated samples the microbial load remained below the detection limit until the 6th storage day. After this storage period a different trend was observed. In sample B (figure 23) a gradual increase of microbial load was noted, although the load did not reach the microbiological acceptability limit during up to 12 days storage. A slight increase of microbial count was also observed in the sample D (figure 23), reaching a maximum value of 3.12 log cfu/g at the end of the 12-day observation period.

The pH of all samples (both cheese and brine) remained almost constant (around 6.0-6.5) during the microbial testing, expect for the control brine that reached values around 5.9-5.5, thus confirming the degradation process beginning to occur.
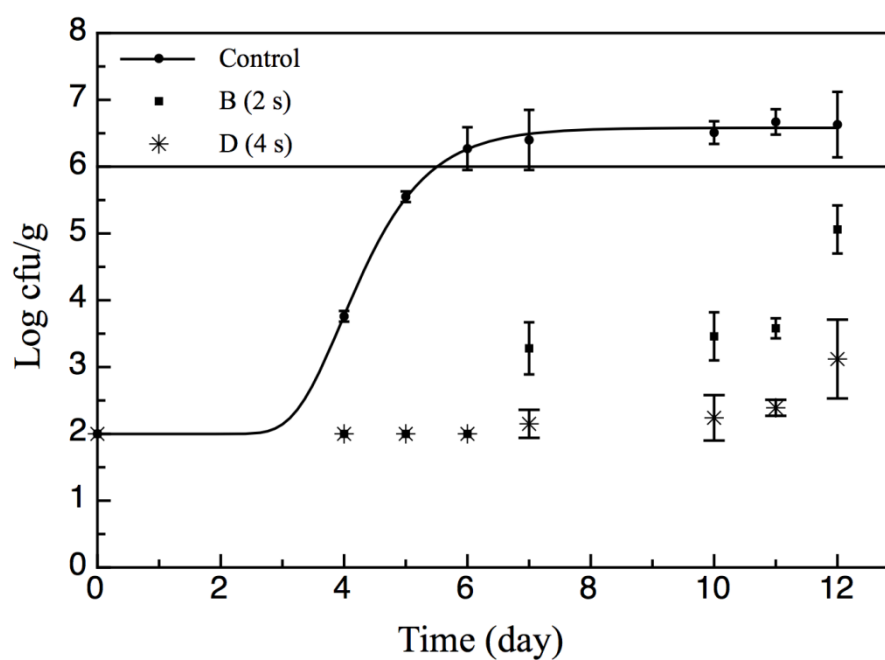
Figure 23 Evolution of *Pseudomonas* spp. population during the refrigerated storage of Fiordilatte cheese exposed to two different High Intensity Light Pulses (HILP) treatments. Symbols: experimental data. Lines: best fits estimates. *Control = untreated Fiordilatte cheese; B = Fiordilatte cheese HILP treated for 2 s; D = Fiordilatte cheese HILP treated for 4 s.*

4.4 Combined Steam-Ultrasound treatment

As reported above, to evaluate the efficacy of combined steam-ultrasound treatment as surface decontamination system on Fiordilatte cheese, the research study was organized in two subsequent phases. In particular, in the first one the effect of this technique was assessed on *P. fluorescens* and Enterobacteriaceae inoculated on cheese surface at two different cell concentrations. These *in-vitro* tests were run to determine the most effective decontamination condition. During the second phase, untreated and steam-ultrasound treated samples were packaged in plastic trays with brine, stored at 9±1 °C and periodically analyzed for microbial quality.

4.4.1 Efficacy of steam-ultrasound treatment on inoculated cheese samples

The effectiveness of treatment on Fiordilatte inoculated at 10^4-10^5 cfu/g was investigated at two different nozzles (H and A). Table 9 shows the microbial count of Enterobacteriaceae and *P. fluorescens* determined in the cheese as function of different treatment times, using the nozzle H (2.4 bar). As one would expect, there is a marked effect of the treatment duration on the survival of spoilage microorganisms. The microbial concentration reduction ranged between 0.8 and 2.36 log cycles. The best result, in the case of Enterobacteriaceae was observed after 6 s of treatment, whereas, in the case of *P. fluorescens* after 2 s. It is important to note that, although this sample (2 s) had the best log reduction, its standard deviation is higher than the others. This implies a greater variability in the sample.

Table 10 shows the same microbial groups as function of different times using the nozzle A (2.7 bar). Also in this case all the treatments provoked a substantial microbial inhibition with a direct proportionality between microbial reduction and treatment time, but best results were obtained with the sample treated for 6 s. Compared to nozzle H (2.4 bar), the nozzle A (2.7 bar) increased the treatment performance. In fact, at the same treatment time (2, 4 and 6 s), a greater steam pressure brought about a greater microbial reduction. This was more evident in the case of Enterobacteriaceae with a decrease much higher than 2 log cycles. An improvement in the efficacy was also observed for *P. fluorescens* (samples treated for 4 and 6 s), with a reduction more than 2 log cycles. The potential use of sound

energy for microbial reduction is well documented, but it appears to have a low lethality at low temperature, the efficacy greatly improves when ultrasound is used in combination with heat (Adekunte et al., 2010; Cameron et al., 2009; Gera & Doores, 2011; James et al., 2007; Boysen & Rosenquist, 2009; Villamiel & de Jong, 2000). Documented studies suggest that microbial inactivation effects of ultrasound are the result of intracellular cavitation. This mechanism causes cell wall perforation, changes of cellular activities, increase of sensitivity to heat and then, cell lysis (Cameron et al., 2009; Ciccolini, et al., 1997; Villamiel & de Jong, 2000). The ultrasound used simultaneously with steam according to the sono-steam system, destroys the protective laminar sub-layer of air that is present around any product. It improves cavitation efficacy, thus promoting rapid heat transfer into the surface of the product within a few seconds (Musavian et al., 2014; Hansen & Larsen, 2007; Boysen & Rosenquist, 2009). Therefore, increasing the pressure, the effectiveness of the cavitation mechanism enhances, promoting a faster heat transfer and consequently a microbial sensitization.

Table 9 Microbial counts of inoculated Fiordilatte cheese exposed to different steam-ultrasound treatment times, using the nozzle H (2.4 bar).

Treatment time (s)	Enterobacteriaceae (log cfu/g)	P. fluorescens (log cfu/g)
Control	5.03 ± 0.05^a	4.78 ± 0.07^a
0.5	4.14 ± 0.14^b	3.71 ± 0.22^b
1	3.91 ± 0.02^c	$3.53 \pm 0.14^{b,c}$
2	$4.04 \pm 0.12^{b,c}$	2.75 ± 0.91^d
4	3.26 ± 0.08^d	$3.04 \pm 0.04^{b,c,d}$
6	2.67 ± 0.20^e	$2.95 \pm 0.10^{c,d}$

[a-e]Data with different letters in each column are significantly different (P<0.05)

Table 10 Microbial counts of inoculated Fiordilatte cheese exposed to different steam-ultrasound treatment times, using the nozzle A (2.7 bar).

Treatment time (s)	Enterobacteriaceae (log cfu/g)	P. fluorescens (log cfu/g)
Control	4.91 ± 0.05^a	4.70 ± 0.04^a
0.5	4.05 ± 0.25^b	3.99 ± 0.25^b
1	3.45 ± 0.02^c	3.10 ± 0.08^c
2	2.59 ± 0.15^d	$2.91 \pm 0.18^{c,d}$
4	2.50 ± 0.57^d	$2.60 \pm 0.63^{c,d}$
6	2.27 ± 0.47^d	2.43 ± 0.38^d

[a-d]Data with different letters in each column are significantly different ($P<0.05$)

As reported beforehand, the efficacy of this combined steam-ultrasound treatment on spoilage microorganisms was also assessed at different microbial concentrations, specifically 10^2-10^3 cfu/g Enterobacteriaceae and 10^3-10^4 cfu/g P. fluorescens. Table 11 shows the behavior of these spoilage microorganisms inoculated on Fiordilatte surface exposed to different treatment times using the nozzle A. Specifically, all the treatment times substantially affected the microbial count of Enterobacteriaceae, thus provoking a total inhibition of microorganisms just with a simple 0.5 s of treatment. The result is in agreement with what reported by Musavian et al., 2015. For P. fluorescens all the treatment times provoked a decrease by about 1 log cycle. Comparing all data of in-vitro tests it's worth noting that steam-ultrasound inactivation efficiency depends on both bacterial specie and microbial concentration (Drakopoullou et al., 2009). Data also highlight that this technique could be particularly effective to reduce the initial cheese contamination, as also reported in other studies carried out on different food (Musavian et al., 2014; 2015). While the sole ultrasound treatment without any steam utilization needs treatment times greater than 5-6 min to eliminate all viable cells (Cameron et al., 2009), the SonoSteam system is able to obtain significant microbial reductions with a very short

treatment time. The simultaneous application of steam and ultrasound can reduce the treatment time and the temperature, provoking the same lethality that could be achieved by applying these technologies separately (Arroyo et al., 2012).

Table 11 Microbial counts of Fiordilatte cheese inoculated at different cell load concentrations after different steam-ultrasound treatments with nozzle A (2.7 bar).

Samples	Treatment time (s)	Enterobacteriaceae (log cfu/g)	*P. fluorescens* (log cfu/g)
Control	-	2.85 ± 0.15^a	3.96 ± 0.05^a
A	0.5	-	$2.26 \pm 0.24^{b,c}$
B	1	-	2.65 ± 0.16^b
C	2	-	2.20 ± 0.17^c
D	4	-	2.10 ± 0.17^c
E	6	-	$2.33 \pm 0.35^{b,c}$

[a-c]Data with different letters in each column are significantly different (P<0.05).

- No detection

4.4.2 Effect of combined steam-ultrasound on Fiordilatte microbiological quality

In the second part of this research work the microbial quality decay kinetic of both treated and untreated Fiordilatte cheese was determined. On the basis of data related to the *in-vitro* tests, the following treatment conditions were used: B (treatment time 1 s; chamber temperature 9.4-9.5 °C; ventilation 35%; 2.7 bar), E (treatment time 6 s; chamber temperature 8.5-9.0 °C; ventilation 70 %; 2.7 bar). All the cheese samples were stored at 9 ± 1 °C and periodically analyzed for 2 weeks. During this observation period the microbial count and pH were monitored.

Growth of spoilage microorganisms as Enterobacteriaceae and *Pseudomonas* spp. was taken into account, being these microbial groups the main responsible bacteria for cheese deterioration (Gammariello et al., 2011; Incoronato et al., 2011; Mastromatteo et al., 2015a,b). As regard Enterobacteriaceae, no proliferation has been found in both untreated and combined steam-ultrasound treated samples, thus suggesting that good hygienic conditions have been adopted during processing of cheese (Del Nobile et al., 2010). Fig. 24 shows the evolution of *Pseudomonas* spp. population in Fiordilatte cheese exposed to increasing combined steam-ultrasound treatment time, compared to untreated samples. The cell concentration of *Pseudomonas* spp. at 10^6 cfu/g has been used as microbial limit for product acceptability, because around this value the alteration of the product may begin to appear (Angiolillo et al., 2014, Gammariello et al., 2011). As can be inferred from data shown in the figure, immediately after the treatment, the initial microbial concentration (3.68 log cfu/g) was drastically reduced below the detection limit ($<10^2$ cfu/g) for both treated samples (B = 1 s, E = 6 s). After 3 days an increase of microbial load was observed in the control sample, until exceeding the acceptability limit after *circa* 4 days of refrigerated storage. A completely different trend was noted for the treated samples B and E where microbial load remained very low and never reached the set microbial acceptability limit.

The pH of all the samples remained almost constant during the entire observation period, around 6.0-6.5, thus suggesting that steam-ultrasound treatment does not affect product pH. A slight pH decrease in the untreated control brine at the end of the storage period was

detected, may be due to the degradation process that took place in the control samples during storage. Therefore, the steam-ultrasound technique was found a very attractive decontamination system to be applied to dairy sector because a few seconds of treatments were effective to control the microbiological quality of mozzarella cheese.
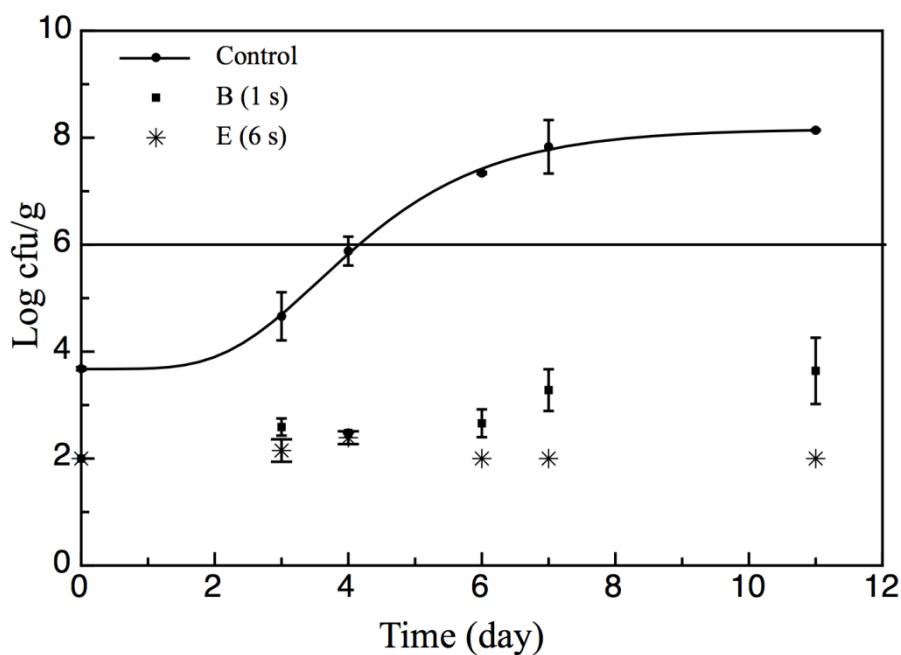
Figure 24 Evolution of *Pseudomonas* spp. population during refrigerated storage of Fiordilatte cheese exposed to two selected steam-ultrasound treatments, compared to untreated samples (control). Symbols: experimental data. Lines: best fits estimates. *Control = untreated Fiordilatte cheese; B = Fiordilatte cheese treated with SonoSteam system for 1 s; E = Fiordilatte cheese treated with SonoSteam system for 6 s.*

4.5 X-rays treatment

4.5.1 X-rays effects on Fiordilatte cheese shelf life (first trial)

To extend the shelf-life of Firdilatte cheese, three different irradiation doses were tested, compared to untreated control. In this study, two replicates were performed. The products packaged into a pouch with its brine were treated with X-ray RS-2400 (Rad Source, USA) and then stored at 9±1 °C to accelerate the phenomena responsible for unacceptability from the consumer. Growth of spoilage microorganisms as Enterobacteriaceae and *Pseudomonas* spp. was taken into account, because these microbial groups are the main responsible bacteria for cheese deterioration (Gammariello et al., 2011; Incoronato et al., 2011; Mastromatteo et al., 2015 a, b). Figure 25 (a, b) shows the evolution during storage of *Pseudomonas* spp. and Enterobacteriaceae count in Fiordilatte cheese irradiated to a dose of 0.5 and 3 kGy compared to untreated control. The cell concentrations of *Pseudomonas* spp. at 10^6 cfu/g and Enterobacteriaceae at 10^5 cfu/g have been used as microbial limit for product acceptability, because around this value was observed that the alteration of the product may begin to appear (Angiolillo et al., 2014, Gammariello et al., 2011). A different trend between untreated and irradiated samples was observed. In particular, in untreated sample (CNT AZ) there was an increase of *Pseudomonas* spp. and Enterobacteriaceae starting from the 1st and the 10th day, respectively, but they never reached the microbiological acceptability limits. For the samples Irrad. 0.5 kGy and Irrad. 3 kGy *Pseudomonas* spp. and Enterobacteriaceae were not detected, though for the sample 0.5 kGy a slight increase of *Pseudomonas* spp. starting from 42nd day, was observed. These results suggest that these spoilage microbial groups seemed to be completely inhibited by irradiation. These results are in agreement with other study carried out on Fiordilatte cheese treated whit electron beam irradiation, suggesting that the spoilage microbial groups are inhibited by the electron irradiation during the entire observation period (Huo et al., 2013).

Compared to other non-thermal technologies, the X-rays treatment had an impact on typical dairy microorganism, in fact an inhibition was observed. The evolution of lactic acid bacteria and lactococci was not similar for treated and untreated samples (data not shown). The increase of irradiation doses influences the microorganism growth. It can be

assumed that the high irradiation dose can inactivate a larger number of microorganisms than the low irradiation dose, leading to the more evident inhibitory effect of sample irradiated with high dose than that with low dose (Huo et al., 2013).
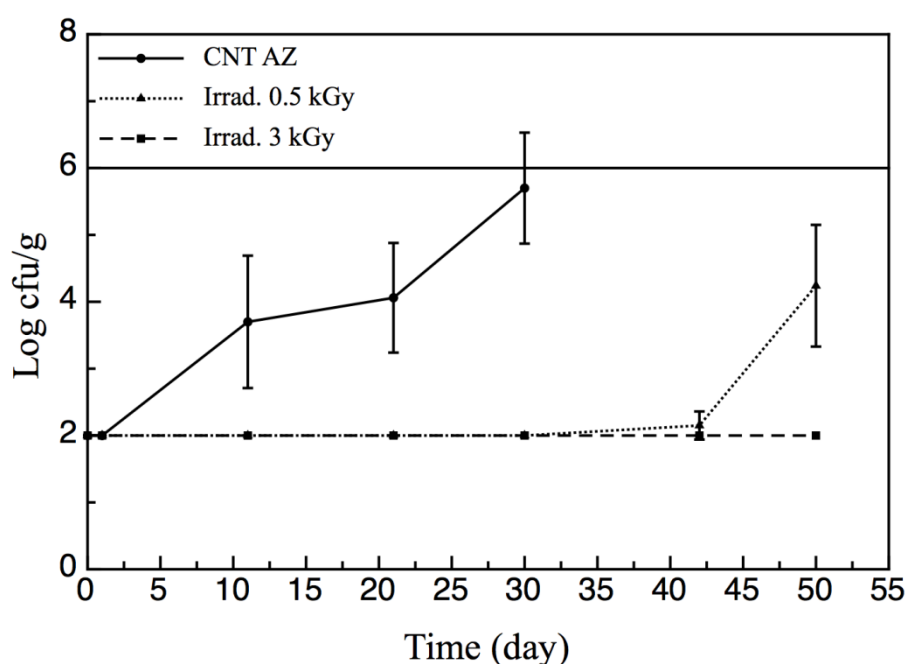
Figure 25 (a) Evolution of *Pseudomonas* spp. population during refrigerated storage of Fiordilatte cheese exposed to two selected X-rays doses, compared to untreated samples (control). *Symbols and lines represent the experimental data. Error bars represent the error with standard deviation. CNT AZ = untreated Fiordilatte cheese; Irrad. 0.5 kGy = Fiordilatte cheese treated with a X-ray dose of 0.5 kGy; Irrad. 3 kGy = Fiordilatte cheese treated with a X-ray dose of 3 kGy.*
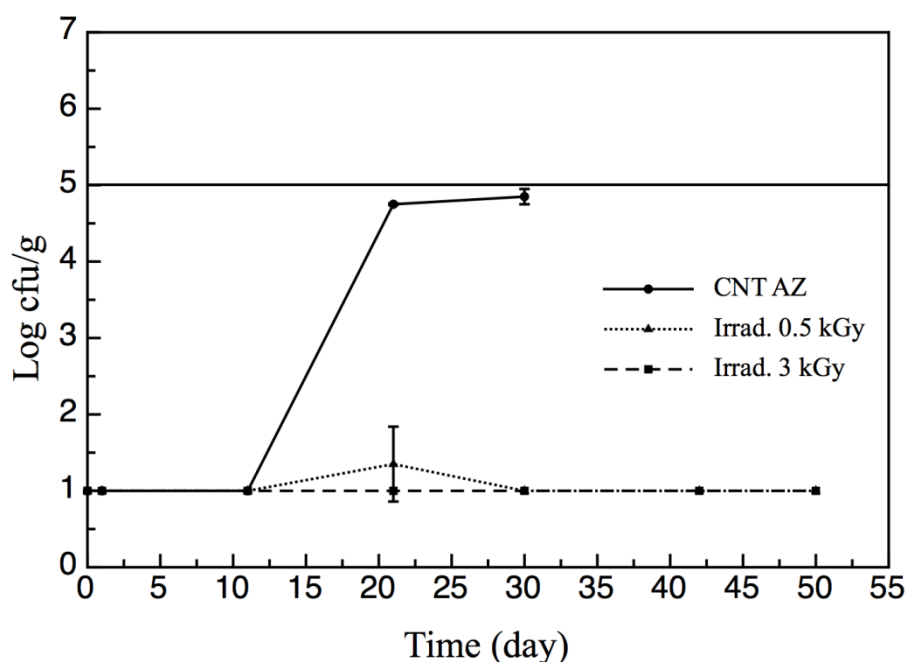
Figure 25 (b) Evolution of Enterobacteriaceae population during refrigerated storage of Fiordilatte cheese exposed to two selected X-rays dose, compared to untreated samples (control). *Symbols and lines represent the experimental data. Error bars represent the error with standard deviation. CNT AZ = untreated Fiordilatte cheese; Irrad. 0.5 kGy = Fiordilatte cheese treated with a X-ray dose of 0.5 kGy; Irrad. 3 kGy = Fiordilatte cheese treated with a X-ray dose of 3 kGy.*

From the sensory point of view a different trend was observed. Figure 26 shows the evolution of the *Overall quality* of Fiordilatte cheese exposed to two X-rays doses. The sensory acceptability limit (SAL) was set in correspondence to a score value equal to 4 on a scale 0-7 in agreement with other studies (Gammariello et al., 2011; Mastromatteo et al., 2015a; 2015b). As expected, during cheese storage, the overall quality score progressively decreased in all the samples. In particular, the control exceded the sensory threshold after *circa* 25 days, the sample 0.5 kGy after *circa* 37 days. While the sample 3 kGy was very near to the sensory limit at the end of the observation period, even if it never reached it. The slight differences among the different irradiated samples were recognized only from the odor point of view. The texture was better maintained, contributing to its acceptability. The same result was obtained by Huo et al., 2013, corroborating the hypothesis that the irradiation does not worsen the sensory characteristics of the cheese.
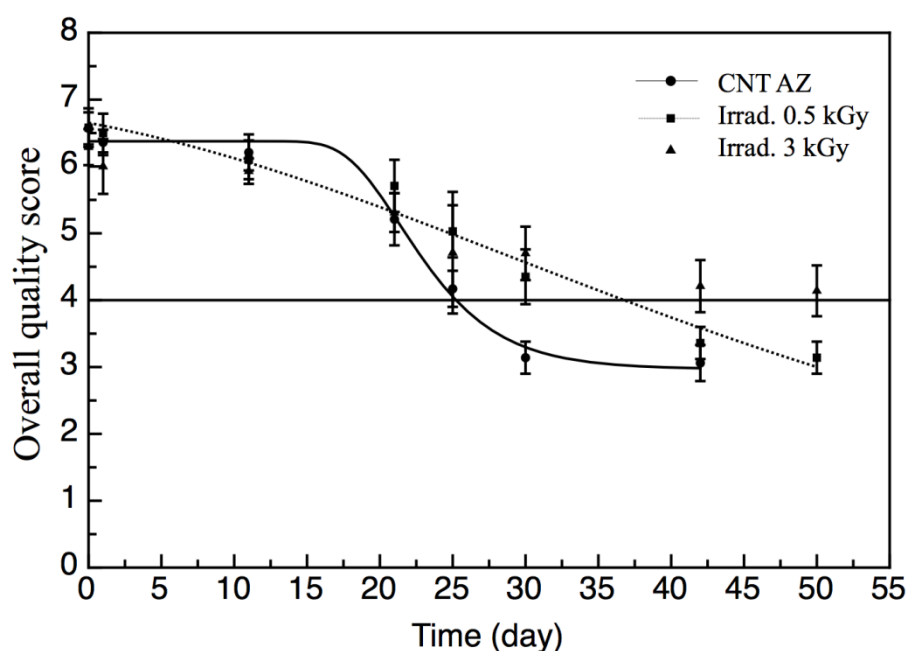
Figure 26 Evolution of the *Overall quality* during the refrigerated storage of Fiordilatte cheese exposed to two selected X-rays dose, compared to untreated samples (control). Symbols: experimental data. Lines: best fits estimates. *CNT AZ = untreated Fiordilatte cheese; Irrad. 0.5 kGy = Fiordilatte cheese treated with X-ray at 0.5 kGy; Irrad. 3 kGy = Fiordilatte cheese treated with X-ray at 3 kGy.*

Fiordilatte shelf-life was calculated as the lowest value among $MAL^{Pseudomonas}$, $MAL^{Enterobacteriaceae}$ and SAL (Conte *et al.*, 2009). For all the samples tested in this study the sensory quality limited the shelf-life (Table 12). Therefore, the best result was noted for the sample 3 kGy with a shelf-life of >50 days, compared to the control that remained acceptable for *circa* 25 days. The other treated sample (Irrad. 0.5 kGy) recorded shelf-life value of *circa* 37 days.

Table 12 Shelf-life (day) of Fiordilatte cheese obtained in the first experimental trial as the lowest value between MAL^{Pseudomonas}, MAL^{Enterobacteriaceae} and SAL

Samples	Microbial quality (day)		Sensory quality (day)	Shelf-life (day)
	MAL^{Pseudomonas}	MAL^{Enterobacteriaceae}	SAL	
CNT AZ	>30	>30	25.2±0.71[a]	25.2±0.71[a]
Irrad. 0.5 kGy	>50	>50	36.8±0.52[b]	36.8±0.52[b]
Irrad. 3 kGy	>50	>50	>50	>50

[a-b] Data with different letters in each column are significantly different (P<0.05)
CNT AZ = untreated Fiordilatte cheese;
Irrad. 0.5 kGy = Fiordilatte cheese treated with X-ray at 0.5 kGy;
Irrad. 3 kGy = Fiordilatte cheese treated with X-ray at 3 kGy.

4.5.2 X-rays effects on Fiordilatte cheese shelf life (second trial)

In agreement with what described above the second replication of this study was performed. Figure 27 (a, b) shows the evolution of *Pseudomonas* spp. and Enterobacteriaceae count in Fiordilatte cheese irradiated to a dose of 0.5, 2 and 3 kGy compared to untreated control. In this case was added an intermediate irradiance dose to evaluate the efficacy of the treatment with a dose near the highest value tested in the first replication. Inasmuch when the dose increased to 2.5 kGy, the ionizing radiation could influence the flavor and the odor of fresh cheese (Huo et al., 2013; Werthein and Procto 1956; Creamer 1976). As expected, for all the samples in general the microbial trend was the same as previously described. In particular, in untreated sample (CNT AZ) *Pseudomonas* spp. started to increase at the 2nd day, but never reached the acceptability limit. While in the irradiated samples (Irrad. 0.5 kGy; Irrad.2 kGy; Irrad.3 kGy) *Pseudomonas* spp. was not detected during the entire observation period, indicating that this spoilage microorganisms seemed to be completely inhibited by irradiation (Huo et al., 2013;). As regard Enterobacteriaceae (figure 27 b), a different trend was observed. In the

untreated sample (CNT AZ) Enterobacteriaceae started to increase after 2 day and reached the acceptability limit after 9 days. While for Irrad. 0.5 kGy and Irrad. 2 kGy samples, there was a slight increase of Enterobacteriaceae around eighth day, but for the following days of monitoring, the microbial load remained below the detection limit until the end of the observation period. On the contrary, the Enterobacteriaceae in the sample Irrad. 3 kGy was not detected during the entire storage period. These results confirm those previously assessed.

Figure 27 (a) Evolution of *Pseudomonas* spp. population during refrigerated storage of Fiordilatte cheese exposed to three selected X-rays doses, compared to untreated samples (control). *Symbols and lines represent the experimental data. Error bars represent the error with standard deviation. CNT AZ = untreated Fiordilatte cheese; Irrad. 0.5 kGy = Fiordilatte cheese treated with X-ray dose of 0.5 kGy; Irrad. 2 kGy = Fiordilatte cheese treated with X-ray dose of 2 kGy; Irrad. 3 kGy = Fiordilatte cheese treated with X-ray dose of 3 kGy.*
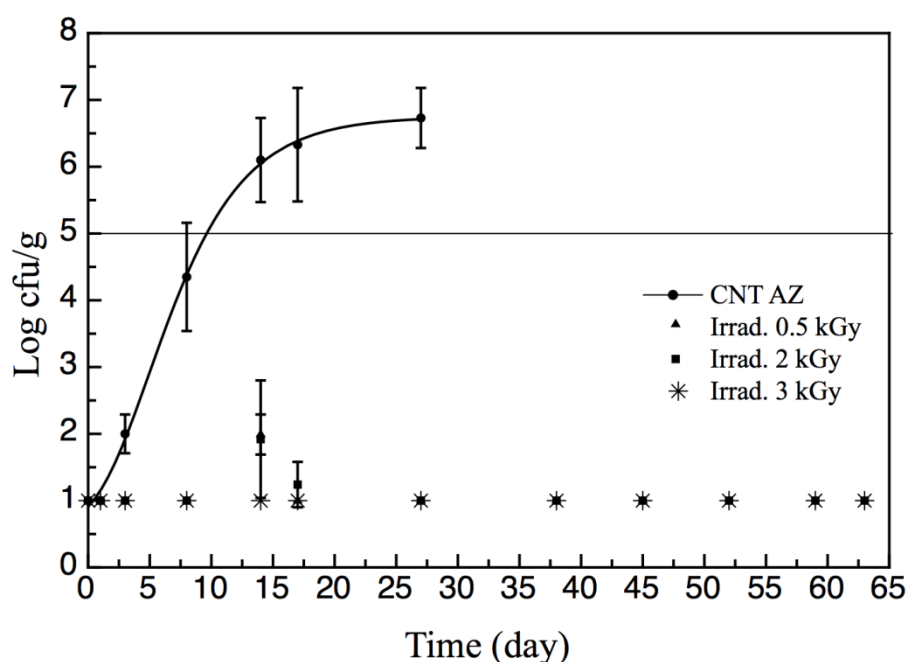
Figure 27 (b) Evolution of Enterobacteriaceae population during refrigerated storage of Fiordilatte cheese exposed to three selected X-rays doses, compared to untreated samples (control). Symbols: experimental data. Lines: best fits estimates. *CNT AZ = untreated Fiordilatte cheese; Irrad. 0.5 kGy = Fiordilatte cheese treated with X-ray dose of 0.5 kGy; Irrad.2 kGy = Fiordilatte cheese treated with X-ray dose of 2 kGy; Irrad.3 kGy = Fiordilatte cheese treated with X-ray dose of 3 kGy*

The effects of X-ray treatment on the sensory properties of Fiordilatte cheese are reported in Figure 28. As expected, during cheese storage, the *overall quality* score progressively decreased in all the samples with different trends. In particular, the untreated sample (CNT AZ) reached the sensory limit (set a value 4) after *circa* 12 days, followed by the sample Irrad. 0.5 kGy that exceeds the limit after *circa* 20 days. Confirming what was observed previously. Instead, the other samples Irrad. 2 kGy and Irrad. 3 kGy exceed the sensory limit after *circa* 44 and 43 days, respectively.
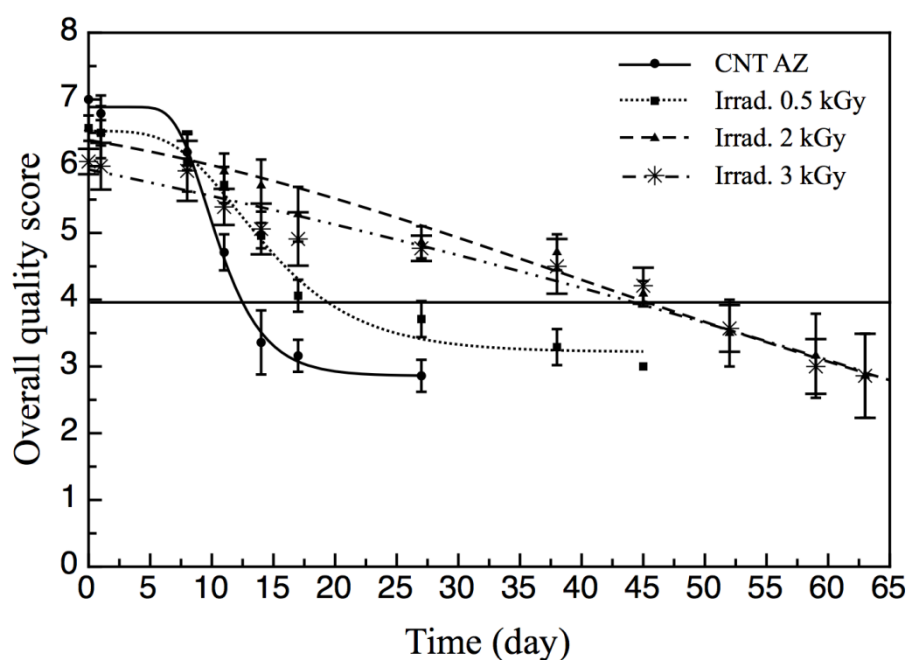
Figure 28 Evolution of the *Overall quality* during the refrigerated storage of Fiordilatte cheese exposed to three X rays doses. Symbols: experimental data. Lines: best fits estimates. *CNT AZ = untreated Fiordilatte cheese; 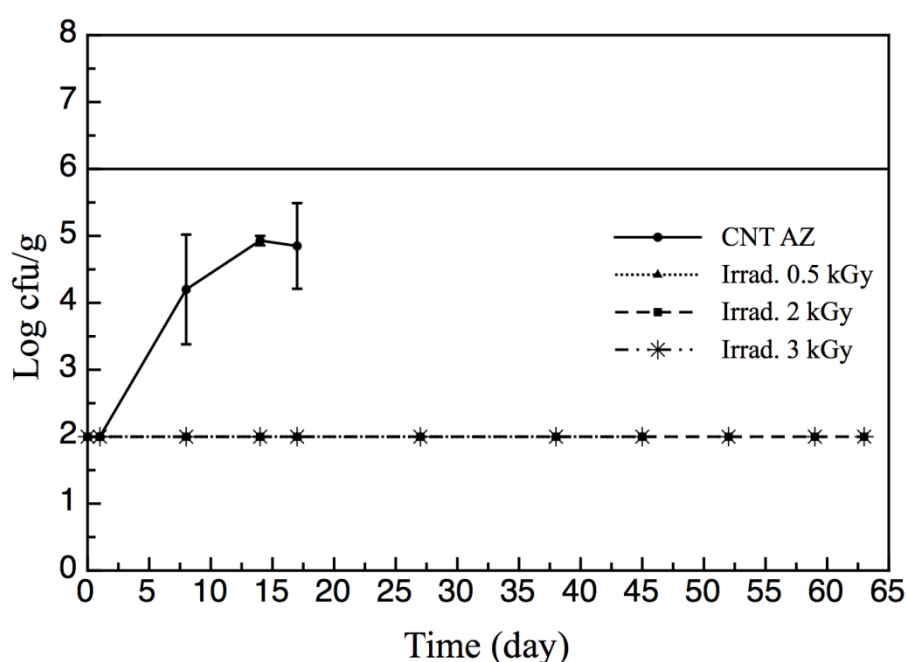Irrad. 0.5 kGy = Fiordilatte cheese treated with X-ray at 0.5 kGy; Irrad. 2 kGy = Fiordilatte cheese treated with X-ray at 2 kGy; Irrad. 3 kGy = Fiordilatte cheese treated with X-ray at 3 kGy.*

Table 13 shows the Fiordilatte shelf life. For the control, the microbial quality limited its shelf-life, as opposed to irradiated samples that the sensory quality limits their shelf-life. As can be seen, the best result was noted for the samples Irrad. 2 and Irrad. 3 kGy with a shelf-life of *circa* 44 and 43 days, respectively. The untreated sample (CNT AZ) remained acceptable for *circa* 10 days, while for the sample Irrad. 0.5 kGy was recorded a shelf-life value of *circa* 20 days.

Table 13 Shelf-life (day) of Fiordilatte cheese obtained in the second experimental trial as the lowest value between $MAL^{Pseudomonas}$, $MAL^{Enterobacteriaceae}$ and SAL

Samples	Microbial quality (day)		Sensory quality (day)	Shelf-life (day)
	$MAL^{Pseudomonas}$	$MAL^{Enterobacteriaceae}$	SAL	
CNT AZ	>27	10.4±0.48[a]	12.4±0.30[a]	10.4±0.48[a]
Irrad. 0.5 kGy	>45	>45	20.2±0.26[b]	20.2±0.26[b]
Irrad. 2 kGy	>63	>63	44.7±0.17[c]	44.7±0.17[c]
Irrad. 3 kGy	>63	>63	43.5±0.28[c]	43.5±0.28[c]

[a-c] Data with different letters in each column are significantly different ($P<0.05$)
CNT AZ = untreated Fiordilatte cheese;
Irrad. 0.5 kGy = Fiordilatte cheese treated with X-ray at 0.5 kGy;
Irrad. 2 kGy = Fiordilatte cheese treated with X-ray at 2 kGy;
Irrad. 3 kGy = Fiordilatte cheese treated with X-ray at 3 kGy.

As shown in Table 12 and 13, samples with irradiation have showed a significant shelf-life increase, respect to the untreated Fiordilatte cheese. This result is related to the inactivation of a large number of spoilage microorganisms, leading to the increases in the shelf life of the investigated Fiordilatte cheese compared to the control. The increase of irradiation doses significantly influenced the shelf-life of the product. In fact, the longer shelf life was obtained when the dose of irradiation was highest. Among the investigated irradiation doses, the best performances were obtained using the dose of 2 and 3 kGy. Therefore, these results, in agreement with a study performed by Huo et al., 2013, suggest that the X-rays may inhibit the growth of *Pseudomonas* spp. and Enterobacteriaceae in Fiordilatte cheese without affecting the sensorial characteristics. Allowing so a prolongation of shelf life of this fresh dairy products.

4.6 Plasma jet treatment

4.6.1 Effects of low temperature plasma on Fiordilatte cheese

Table 14 (a, b) reports the behavior of microbial population. As shown, the different treatment conditions of plasma had an antimicrobial effect against inoculum of *Pseudomonas fluorescens* and *P. putida*, regardless of the type of plasma treatments applied on the surface or immersed in the sample. However, the same treatments applied to Fiordilatte cheese highlighted that no effects were recorded with a real product application (Table 15), probably due to the more complicated food matrix, the presence of fat and protein in the Fiordilatte cheese that could have a protective action against microorganisms. One of the factors that influence the effectiveness of plasma is the type of food which is being treated (Song et al., 2009), but also the plasma characteristics, the type of microorganisms and the initial microbial load (Laroussi, M. and Leipold, F., 2004; Moisan et al., 2002).

Table 14 (a) Behavior of microbial count (*P. fluorescens* and *P. putida*) in samples treated with plasma jet immersed.

Sample	Treatment	Mix *Pseudomonas* log cfu/g
Control	Untreated	4.31 ± 0.29^a
PLASMA.1	He 15min	3.18 ± 0.00^b
PLASMA.2	He+O_2 (0.15 %) 15min.	<DL
PLASMA.3	He+O_2 (0.25 %) 15min	<DL
PLASMA.4	He+O_2 (0.40 %) 15min	<DL

[a-b]For each parameter means with the same letter are not significantly different (p <0.05).

DL: Detection limit 2.00 cfu/g

Table 14 b Behavior of microbial count (*P. fluorescens* and *P. putida*) in samples treated with plasma jet on surface.

Sample	Treatment	Mix *Pseudomonas* log cfu/g
Control	Untreated	4.31±0.29[a]
PLASMA.5	He 15min	2.50±0.12[b]
PLASMA.6	He+O_2 (0.15 %) 15min	<DL
PLASMA.7	He+O_2 (0.25 %) 15min	<DL
PLASMA.8	He+O_2 (0.40 %) 15min	<DL
PLASMA.9	He+O_2 (0.55 %) 15min	<DL

[a-b]Data in column with different letters are significantly different ($P<0.05$).
DL: Detection limit 2.00 cfu/g.

Table 15 Plasma jet effects on spoilage microorganisms of Fiordilatte cheese.

Sample	Treatment	*Pseudomonas* spp. log cfu/g	Enterobacteriaceae log cfu/g
Control	Untreated	6.86±0.67[a]	5.24±0.56[a]
CAMP.1	He+O_2 0.40% (jet on surface) 60min	6.71±0.65[a]	5.34±0.44[a]
CAMP.2	He+O_2 0.55% (jet on surface) 60min.	6.98±0.55[a]	5.72±0.31[a]
CAMP.3	He+O_2 0.40% (jet immersed) 60min	6.93±0.39[a]	5.70±0.71[a]
CAMP.4	He+O_2 0.55% (jet immersed) 60min	6.98±0.46[a]	5.44±0.70[a]

[a]Data in column with different letters are significantly different ($P<0.05$).

The results obtained highlighted that no antimicrobial efficacy was found, even though promising results were recorded on inoculated saline solution samples. It was also noted that the gas utilized as carrier plays a key role in the efficacy of the treatment.

5.　Conclusions

The adoption of non-thermal technologies for food preservation could represent an alternative strategy. UV-C light, pulsed light, high intensity light pulses, combined steam-ultrasound and X-ray treatments are a promising methods to inactivate Fiordilatte cheese spoilage microorganisms and prolong the shelf life of these fresh dairy products. Each preservation strategy has various factors that can limit the efficacy of the treatment. But the composition, the shape and the irregular surface of Fiordilatte cheese play an important role in the effectiveness of these treatments. The main conclusions of this work are:

1. UV-C light was assessed for the first time on Fiordilatte cheese. The efficacy of this treatment is very interesting because a shelf-life extension was recorded in treated samples. From the preliminary test with inoculated samples, UV-C light was demonstrated to decontaminate surface of Fiordilatte cheese. The increase in UV-C light fluence did not always increase the decontamination efficiency, thus suggesting that a more complex relationship than direct proportionality exists between UV-C light dose and antimicrobial effects on the product. The 80% shelf-life extension was achieved by selecting adequate processing conditions of exposure of Fiordilatte cheese to UV-C light (6.0 kJ/m^2). Further research is still necessary to better optimize UV-C light parameters that allow recording significant shelf-life extension without provoking undesired quality changes. Specifically, the efficacy of UV-C exposure between 60 and 300 s could be better investigated.

2. PL treatment may allow a reduction of microbial growth, especially immediately after the treatment. From the preliminary results, performed on native spoilage microflora (Fiordilatte cheese were treated with PL an electric potential of 1000, 2000, 2500 and 3000 V) *Pseudomonas* spp. was not inactivated. Probably due to the high initial contamination levels (10^6 cfu/g) on Fiordilatte cheese that reduce

the decontamination efficacy of this treatment. On the contrary for Enterobacteriaceae a better result was observed. All treatments were able to reduce the initial microbial contamination below the detection limit with *circa* 4 log cycles of microbial reduction. Significant reductions of 1.27 and 2.06 log cycle were achieved for *Pseudomonas* inoculated on Fiordilatte cheese. These results showed that the microbial count for both treatment conditions (2000 V and 3000 V) decreased with the increase in light fluence. During the shelf life test the PL efficacy on *Pseudomonas* spp. was mainly observed within the first two days of storage. After this time all the treated samples reached microbial populations similar to that recorded in the control cheese, that becames unacceptable just after few days (*circa* 2 day). Furthermore, data confirm that Enterobacteriaceae are more photo-sensitive than *Pseudomonas* spp. These results confirm that the PL treatment exerts an initial germicidal activity but it is not able to inhibit microbial growth during storage. In this case there was not a significant shelf-life prolongation compared to untreated control. This finding could be explained considering that PL only acts on the product surface and does not significantly penetrate into the food. Further research is still necessary to optimize the application of PL and to continue research to better allow shelf-life extension without provoking undesired changes on Fiordilatte cheese quality.

3. The efficacy of HILP treatment is very interesting because a microbial reduction was observed in treated samples. From the preliminary test it was observed that in a transparent liquid matrix (Maximum Recovery Diluent, MRD) there was a significant microbial inactivation just with 2 s of treatment (fluence 7.02 J/cm^2). These results show that transparency of the media allowed successful microbial inactivation using this technology. In the case of inoculated Fiordilatte, a great microbial reduction (about 1 log cycle) was observed until 4 s of exposures, even if a lower microbial inactivation was noticed compared to that obtained in the transparent liquid. For *Pseudomonas* spp. in particular, the microbiological acceptability limit (10^6 cfu/g) was never reached after 2 weeks of storage, thus suggesting that the technique is very promising for prolonging product shelf-life. HILP treatment is able to control the microbial growth and may be considered a

promising way to decontaminate the surface of Fiordilatte cheese. The short treatment times lend itself to application in high volume production lines. Further research is still necessary to optimize the application of HILP and to continue research to better assessed the effects on the total cheese quality.

4. In the study performed with combined steam-ultrasound treatment all data of *in-vitro* tests highlighted that this technique could be particularly effective to reduce the initial cheese contamination, with a reduction ranged between 0.8 and more than 2 log cycles. Its inactivation efficacy depends on both bacterial specie and microbial concentration. Furthermore, the use of different nozzles highlighted that there was an increase of the treatment performance, with a greater microbial reduction using the nozzle at the highest pressure level (A=2.7-3.4 bar). In fact, at the same treatment time (2, 4 and 6 s), a greater steam pressure brought about a greater microbial reduction. This was more evident in the case of Enterobacteriaceae with a decrease much higher than 2 log cycles. An improvement in the efficacy was also observed for *P. fluorescens* (samples treated for 4 and 6 s), with a reduction more than 2 log cycles. This study also showed that a rapid combined steam-ultrasound treatment was effective to control the microbial quality of Fiordilatte cheese. In particular, immediately after the treatment, the initial microbial concentration (3.68 log cfu/g) was drastically reduced below the detection limit ($<10^2$ cfu/g) for both treated samples (B = 1 s, E = 6 s). The control samples exceed the acceptability limit after *circa* 4 days, while for the treated samples B and E the microbial load remained very low and never reached the set microbial acceptability limit. The treatment duration is a very important issue for any industrial application because it also could affect the sensory quality of food. The very short time necessary to apply this technique with success allows suggesting that no relevant changes in sensory quality can be expected. Anyhow, a deeper investigation on sensory quality should be also carried out. In addition, further work could be also made to assess the effectiveness of this preservation strategy on cheese at various contamination levels to verify the best combination steam-ultrasound to reach desired shelf-life values.

5. X-rays are a non-thermal technology that have shown promise for reducing spoilage bacteria on Fiordilatte cheese. In this study the potential use of X-ray radiation to ensure the safety and extend the shelf life of Fiordilatte cheese was investigated. Under the tested conditions (0.5, 2 and 3 kGy), the efficacy of the X-ray treatment against *Pseudomonas* spp. and Enterobacteriaceae increased with increased the X-ray doses. The results show that X-rays are able to increase significantly the shelf life of all irradiated Fiordilatte cheeses. Among the investigated irradiation doses, the best performances were obtained using the dose of 2 and 3 kGy with a shelf life of 44 and 43 days, respectively. On the contrary the untreated control (CNT AZ) remained acceptable for *circa* 10 days. Irradiation did not affect the sensory characteristics of Fiordilatte cheese, however a slight differences among the different irradiated samples were recognized only from the odor point of view. The texture was better maintained, contributing to its acceptability. This confirms that the irradiation may reduce the growth of spoilage microorganisms such as *Pseudomonas* spp. and Enterobacteriaceae without affecting the sensorial characteristics of the product. The possibility to treat already packaged samples, allows to overcome the problem of post-processing contamination and this is a very important consideration for any industrial application. However, further research is still necessary to optimize the application of this technology without provoking undesirable sensorial quality changes.

5.1 Recommendations for future work

Results obtained in this PhD thesis can confirm that non-thermal technologies are promising alternative methods to extend the shelf life of Fiordilatte cheese. Each preservation strategy, in different way, exerted its efficacy. After the various treatments, there was an inactivation of the main responsible bacteria for cheese deterioration (*Pseudomonas* spp. and Enterobacteriaceae), while the sensory acceptance is maintained. Toxic compounds seem not be generated. However, further research need to be done about the nutritional aspects and any nutritional losses. The scale-up of the technologies will require an optimization of the different parameters. Therefore, in the future, more studies could be carried out to determine the synergistic effects of combining these non-thermal technologies each others or with High Pressure treatments, Pulsed Electric Field (PEF) or with natural preservatives applied during processing.

References

Adekunte, A., Valdramidis, V.P., Tiwari, B.K., Slone, N., Cullen, P.J., Donnell, C.P., & Scannell, A. (2010). Resistance of *Cronobacter sakazakii* in reconstituted powdered infant formula during ultrasound at controlled temperatures: A quantitative approach on microbial responses. *International Journal of Food Microbiology*, 142, 53-59.

Akram, K., Ahn, J.J., Yoon, S.R., Kim, G.R., & Kwon, J.H. (2012). Quality attributes of *Pleurotus eryngii* following gamma irradiation. *Postharvest Biology and Technol*ogy, *66*, 42–47.

Allende, A., McEvoy, J. L., Luo, Y. G., Artes, F., & Wang, C. Y. (2006). Effectiveness of two-side UV-C treatments in inhibiting natural microflora and extending the shelf-life of minimally processed "Red Oak Leaf" lettuce. *Food Microbiology*, 23, 241-249.

Allende, A., Tomas-Barberan, F. A., & Gil, M. I. (2006b). Minimal processing for healthy traditional foods. *Trends in Food Science & Technology*, 17, 513–519.

Altieri, C., Scrocco, C., Sinigaglia, M., & Del Nobile, M. A. (2005). Use of chitosan to prolong mozzarella cheese shelf life. *Journal Dairy Science*, 88, 2683–2688.

Alzamora, S. M., Guerrero, S. N., Schenk, M., Raffellini, S., & López-Malo, A. (2011). Inactivation of microorganisms. *Ultrasound technologies for food and bioprocessing*, 321–343.

Anderson, J. G., Rowan, N. J., MacGregor, S. J., Fouracre, R. A., & Farish, O. (2000). Inactivation of food-borne enteropathogenic bacteria and spoilage fungi using pulsed-light. *IEEE Transactions on Plasma Science*, 28, 83–88.

Angiolillo, L., Conte, A., Faccia, M., Zambrini, A. V., & Del Nobile, M. A. (2014). A new method to produce symbiotic Fiordilatte cheese. *Innovative Food Science and Emerging Technologies*, 22, 180-187.

Arena, V. (1971). Ionizing radiation and life, Mosby, St. Louis, MO.

Arjunan, K.P. & Clyne, A.M. (2011). Hydroxyl radical and hydrogen peroxide are primarily responsible for dielectric barrier discharge plasma induced angiogenesis. *Plasma Process Polymers*, 8, 1154-1164.

Arroyo, C., Cebrián, G., Pagán, R., & Condón, S. (2012). Synergistic combination of heat and ultrasonic waves under pressure for *Cronobacter sakazakii* inactivation in apple juice. *Food Control*, 25, 342-348.

Awad T. S., Moharram H. A., Shaltout O. E., Asker D., & Youssef, M. M. (2012). Applications of ultrasound in analysis, processing and quality control of food: A review. *Food Research International,* 48, 410-427.

Ball, D.W. (2007). The electromagnetic spectrum: a history. *Spectroscopy*, 22, 14-17.

Bandla, S., Choudhary, R., Watson, D. G., & Haddock, J. (2012). UV-C treatment of soymilk in coiled tube UV reactors for inactivation of *Escherichia coli* W1485 and *Bacillus cereus* endospores, *LWT – Food Science and Technology*, 46, 71–76.

Bank, H. L., John, J., Schmehl, M. K., & Dracht, R. K. (1990). Bactericidal effectiveness of modulated UV light. *Applied and Environmental Microbiology*, 56, 3888-3889.

Barbosa-Canovas, G. V., Pothakamury, U. R., Palou, E., & Swanson, B. G. (1998). Nonthermal preservation of foods. New York: Marcel Dekker, 139–161.

Barbosa-Canovas, G.V., Gongora-Nieto, M.M., Pothakamury, U.R., & Swanson, B.G., (1999). Preservation of Foods with Pulsed Electric Fields. Academic Press, San Diego.

Baruzzi, F., Lagonigro, R., Quintieri, L., Morea, M., & Caputo, L., (2012). Occurrence of non-lactic acid bacteria populations involved in protein hydrolysis of cold stored high moisture Mozzarella cheese. *Food Microbiol*ogy. 30, 37-44.

Basaran N., Quintero-Ramos A., Moake M. M., Churey J. J., & Worobo R. W. (2004). Influence of apple cultivars on inactivation of different strains of *Escherichia coli* O157:H7 in apple cider by UV irradiation. *Application Enviroment Microbiology,* 70, 6061-5.

Bermudez-Aguirre, D., & Barbosa-Canovas, G.V. (2010). Processing of soft Hispanic cheese (Queso Fresco) using thermo-sonicated milk: A study of physicochemical characteristics and storage life. *Journal of Food Science*, 75, 548-558.

Bermudez-Aguirre, D., Corradini, M.G., Mawson, R., & Barbosa-Canovas, G.V. (2009a). Modeling the inactivation of *Listeria innocua* in raw whole milk treated under thermo-sonication. *Innovative Food Science and Emerging Technologies*, 10, 172-178.

Bermudez-Aguirre, D., Mawson, R., Versteeg, K. and Barbosa-Canovas, G.V. (2009b). Composition parameters, physical-chemical characteristics and shelf-life of whole milk after thermal and thermo-sonication treatments. *Journal of Food Quality*, 32, 283-302.

Bevilacqua, A., Corbo, M. R., & Sinigaglia, M. (2007). Combined effects of modified atmosphere packaging and thymol for prolonging the shelf life of caprese salad. *Journal of Food Protection*, 70, 722-728.

Bialka, K.L., & Demirci, A., (2008). Efficacy of pulsed UV-light for the decontamination of *Escherichia coli* O157:H7 and *Salmonella* spp. on raspberries and strawberries. *Journal of Food Science*, 73, 201–207.

Bintsis, T., Litopoulou-Tzanetaki, E., & Robinson, R. K. (2000). Existing and potential application of ultraviolet light in the food industry- a critical review. *Journal of the Science of Food and Agriculture*, 90, 637-645.

Birk, T., & Knøchel, S., (2009). Fate of food-associated bacteria in pork as affected by marinade, temperature, and ultrasound. *Journal of Food Protection*, 72, 549–555.

Bolumar T., Andersen M.L., & Orlien V., (2011). Antioxidant active packaging for chicken meat processed by high pressure treatment. *Food Chemistry,* 129, 1406-1412.

Boysen, L., & Rosenquist, H. (2009). Reduction of thermotolerant *Campylobacter* species on broiler carcasses following physical decontamination at slaughter. *Journal of Food Protection*, 72, 497-502.

Brocklehurst, T.H., & Lund, B.N. (1985): Microbiological changes in cottage cheese varieties during storage at +7 °C. *Food microbiology*, 2, 207-233.

Buckin, W., Kudryushov, E., & O'Driscoll, B. (2002). High-resolution ultrasonic spectroscopy for material analysis. American Laboratory, 28–31.

Butz, P., & Tauscher, B., (2002). Emerging technologies: chemical aspects. *Food Research International* 35, 279-284.

Cameron, M., McMaster, L.D., & Britz, T.J. (2008). Electron microscopic analysis of dairy microbes inactivated by ultrasound. *Ultrasonics Sonochemistry*, 15, 960–964.

Cameron, M., McMaster, L.D., & Britz, T.J. (2009). Impact of ultrasound on dairy spoilage microbes and milk components. *Dairy Science and Technology*, 89, 83-98.

Caminiti I.M, Noci F, Morgan D.J., Cronin D.A., & Lyng J.G., (2012). The effect of pulsed electric fields, ultraviolet light or high intensity light pulses in combination with manothermosonication on selected physico-chemical and sensory attributes of an orange and carrot juice blend. *Food and Bioproducts Processing*, 90, 442-448.

Caminiti, I.M., Noci, F., Muñoz, A., Whyte, P., Morgan, D.J., Cronin, D.A., Lyng, J.G., (2011). Impact of selected combinations of non-thermal processing technologies on the quality of an apple and cranberry juice blend. *Food Chemistry*,124, 1387-1392.

Cantoni, C., Milesi, S., & Iacumin, L. (2006). *Kocuria rhizophila, Pseudoclavibacter helvolus* e *Pseudomonas putida* quali germi alteranti i prodotti lattiero-caseari. *Industrie Alimentari*, 1261-1263.

Cantoni, C., Stella, S., & Cozzi, M., (2003). Blue colouring of Mozzarella cheese. *Industrie Alimentari*, 42, 840-843.

Caputo, L., Quintieri, L., Bianchi, D. M., Decastelli, L., Monaci, L., Visconti, A., & Baruzzi, F. (2015). Pepsin-digested bovine lactoferrin prevents Mozzarella cheese blue discoloration caused by *Pseudomonas fluorescens*. *Food Microbiology*. 46, 15-24.

Carascosa, C., Millán, R., Jaber, J. R., Lupiola, P., de Rosario-Quintana, C., Mauricio, C., & Sanjuán, E. (2015). Blue pigment in fresh cheese produced by *Pseudomonas fluorescens*. *Food Control*. 54, 95-102.

Chemat, F., Zill-e-Huma, & Khan, M. K. (2011). Applications of ultrasound in food technology: Processing, preservation and extraction. *Ultrasonics Sonochemistry*, 18, 813-835.

Chen, C.,Wolle, D., & Sommer, D. (2009). Mozzarella. In S. Clark, M. Costello, M. Drake, & F. Bodyfelt (Eds.), The Sensory Evaluation of Dairy Products (pp. 468–469) (2nd ed.). Dordrecht, the Netherlands: Springer Science+Business Media.

Choi, M.-S., Cheigh, C.-I., Jeong, E.-A., Shin J.-K., & Chung, M.-S. (2010). Nonthermal sterilization of *Listeria monocytogenes* in infant foods by intense pulsed-light treatment, *Journal of Food Engineering*, 97, 504–509.

Choudhary, R. & Bandla, S. (2012). Ultraviolet pasteurization for food industry. *International Journal of Food Science and Nutrition Engineering*, 2, 12-15.

Choudhary, R., Bandla, S., Watson, D.G. Haddock, J., Abughazaleh, A., & Bhattacharya B. (2011). Performance of coiled tube ultraviolet reactors to inactivate *Escherichia coli* W1485 and *Bacillus cereus* endospores in raw cow milk and commercially processed skimmed cow milk, *Journal of Food Engineering*, 107: 14–20.

Ciccolini, L., Taillandier, P., Wilhem, A. M., Delmas, H., & Strehaiano, P. (1997). Low frequency thermo-ultrasonication of *Saccharomyces cerevisiae* suspensions: effect of temperature and of ultrasonic power. *Chemical Engineering Journal*, 65, 145-149.

Cilliers, F. P., Gouws, P. A., Koutchma, T., Engelbrecht, Y., Adriaanse, C., & Swart, P. (2014). A microbiological, biochemical and sensory characterisation of bovine milk

treated by heat and ultraviolet (UV) light for manufacturing Cheddar cheese. *Innovative Food Science and Emerging Technologies,* 23, 94-106.

Conte, A., Gammariello, D., Di Giulio, S., Attanasio, M., & Del Nobile, M. A. (2008). Active coating and modified atmosphere packaging to extend the shelf life of Fior di latte cheese. *Journal Dairy Science*, 9, 887-894.

Conte, A., Scrocco, C., Sinigaglia, M., & Del Nobile, M. A. (2007). Innovative active packaging system to prolong the shelf life of Mozzarella cheese. *Journal Dairy Science*, 90, 2126–2131.

Coppola, S., Fusco, V., Andolfi, R., Aponte, M., Blaiotta, G., Ercolini, D., & Moschetti, G., (2006). Evaluation of microbial diversity during the manufacture of fior di latte di agerola, a traditional raw milk pasta-filata cheese of the Naples area. *Journal of Dairy Research*, 73, 264–272.

Corbo, M. R., Del Nobile, M. A., & Sinigaglia, M. (2006). A novel approach for calculating shelf-life of minimally processed vegetables. *International Journal Food Microbiology*, 106, 69-73.

Corradini, C., & Innocente, N. (2002). Parametri chemiometrici e descrittori sensoriali del Montasio DOP. Notiziario Ersa, 4, 43-45.

Crawford, L. M., & Ruff, E. H., (1996). A review of the safety of cold pasteurization through irradiation. *Food Control*, 7, 87-97.

Cruz, R. M. S., Vieira, M. C. & Silva, C. L. M. (2006). Effect of heat and thermosonication treatments on peroxidase inactivation kinetics in watercress (*Nasturtium officinale*). *Journal of Food Engineering*, 7, 8-15.

D'Amico, D. J., Silk, T. M., Wu, J. R. & Guo, M. R. (2006). Inactivation of microorganisms in milk and apple cider treated with ultrasound. *Journal of Food Protection*, 69, 556-563.

Davies, M. J. (2003). Singlet oxygen-mediated damage to proteins and its consequences. Biochemical and Biophysical Research Communications, 305, 761-770.

De Angelis, M., de Candia, S., Calasso, M.P., Faccia, M., Guinee, T.P., Simonetti, M.C., & Gobbetti, M. (2008). Selection and use of autochthonous multiple strain cultures for the manufacture of high-moisture traditional mozzarella cheese. *International Journal of Food Microbiology*, 125, 123–132.

De Angelis, M., & Gobbetti, M. (2011). Pasta-filata cheeses: traditional Pasta-filata cheese. In: Fuquay, J.W. (Ed.), Encyclopedia of Dairy Sciences, 2nd ed. vol. 1. Elsevier, New York, USA, pp. 745–752.

de Candia, S., De Angelis, M., Dunlea, E., Minervini, F., McSweeney, P. L. H., Faccia, M., & Gobbetti, M. (2007). Molecular identification and typing of natural whey starter cultures and microbiological and compositional properties of related traditional mozzarella cheeses. *International Journal of Food Microbiology*. 119, 182–191.

de Capdeville, G., Wilson, C. L., Beer, S. V., & Aist, J. R. (2002). Alternative disease control agents induce resistance to blue mold in harvested "red delicious" apple fruit. *Phytopathology,* 92, 900-908.

De Gennaro, L., Cavella, S., Romano, R. & Masi, P. (1999). The use of ultrasound in food technology I: Inactivation of peroxidase by thermosonication. *Journal of Food Engineering*, 39, 401-407.

De Jonghe, V., Coorevits, A., Van Hoorde, K., Messens, W., Van Landschoot, A., De Vos, P., & Heyndrickx, M., (2011). Influence of storage conditions on the growth of *Pseudomonas* species in refrigerated raw milk. *Applied and Environmental Microbiol*ogy, 77, 460-470.

Del Nobile, M. A., Gammariello, D., Conte, A., & Attanasio M. (2009). A combination of chitosan, coating and modified atmosphere packaging for prolonging Fior di latte cheese shelf life. *Carbohydrate polymers*, 78,151-156.

Del Nobile, M. A., Gammariello, D., Di Giulio, S., & Conte, A. (2010). Active coating to

prolong the shelf life of Fior di latte cheese. *Journal of Dairy Research*, 77, 50-55.

Demirdŏven, A., & Baysal, T. (2009). The use of ultrasound and combined technologies in food preservation. *Food Reviews International*, 25, 1-11.

Diehl J. F. (2002). Food irradiation: past, present and future. *Radiation Physics and Chemistry*, 63, 211-215.

Dionísio, A. P., Gomes, R. T., & Oetterer, M. (2009). Ionizing Radiation effects on food vitamins-a review. *Brazilian Archives of Biology and Technology - An International Journal*, 52, (5), 1267-1278.

Dolatowski, Z.J., & Stasiak, D. M. (2002). Bacterial contamination of meat and meat products after ultrasound treatment. *Acta Science of Poultry and Technology Alimentary*, 1, 55–65.

Drakopoullou, S., Terzakis, S., Fountoulakis, M. S. Mantzavinos, D., & Manios, T. (2009). Ultrasound-induced inactivation of gram-negative and gram-positive bacteria in secondary treatment municipal water. *Ultrasonics Sonochemistry*, 16, 629-634.

Dunn, J. (2000). Pulsed-light disinfection of water and. sterilization of blow/fill/seal manufactured aseptic pharmaceutical products. In *Disinfection, Sterilization, and Preservation* (ed. S.S. Block). Lippincott Williams and Wilkins, USA.

Dunn, J. E., Clark, R. W., Asmus, J. F., Pearlman, J. S., Boyer, K., Painchaud, F., & Hofmann G. A. (1991). Methods for preservation of foodstuffs. US patent number 5034235.

Dunn, J., Ott, T., & Clark,W. (1995). Pulsed-light treatment of food and packaging. *Food Technology,* 49, 95-98.

Earnshaw, R. G. (1998). Ultrasound: A new opportunity for food preservation, In: Povey, M.J.W and Mason, T.J., Eds., *Ultrasound in Food Processing*, Blackie Academic and Professional, London, 183-192.

Earnshaw, R. G., Appleyard, J. & Hurst, R. M. (1995). Understanding physical inactivation processes: Combined preservation opportunities using heat, ultrasound and pressure. *International Journal of Food Microbiology*, 28, 197-219

Ehlbeck, J., Schnabel, U., Polak, M., Winter, J., Woedtke, T., Brandenburg, R., Hagen, T. & Weltmann, K. D. (2011). Low temperature atmospheric pressure plasma sources for microbial decontamination. *Journal of Physics D: Applied Physics*, 44, 013002.

Elmnasser, N., Guillou, S., Leroi, F., Orange, N., Bakhrouf, A., & Federighi, M. (2007). Pulsed-light system as a novel food decontamination technology: A review. *Canadian Journal of Microbiology*, 53, 813–821.

Eneroth, A., Ahrne, S., & Molin, G. (2000). Contamination of milk with Gram negative spoilage bacteria during filling of retail containers. *International Journal of Food Microbiology 57*, 99-106.

Ercan, S. S., & Soysal, C. (2013). Use of ultrasound in food preservation. *Natural Science*, 5, 5-13.

Escalona, V. H., Aguayo, E., Martinez-Hernandez, G. B., & Artes, F. (2010). UV-C doses to reduce pathogen and spoilage bacterial growth in vitro and in baby spinach. *Postharvest Biology and Technology*, 56, 223-231.

Evrendilek, G. A., Zhang, Q. H., & Richter, E. R. (2004). Application of Pulsed Electric Fields to Skim Milk inoculated with *Staphylococcus aureus*. *Biosystems Engineering*, 87, 137-144.

Faccia, M., Trani, A., & Di Luccia, A, (2009). Short communication: relationships between milk quality and acidification in the production of table mozzarella without starters. *Journal of Dairy Science*, 92, 4211–4217.

Faccia, M., Trani, A., Loizzo, P., Gambacorta, G., Ruppi, G., & Di Luccia, A. (2011). Shelf-life della mozzarella vaccina conservata in soluzioni saline: primi risultati. *Scienza e Tecnica Lattiero-Casearia*, 62, 167-171.

Farkas, J. (2006). Irradiation for better food. *Trends in Food Science & Technology*, 17, 148-152.

Fellows, P. (2000). Food Processing Technology: Principles and Practice, 2nd ed. CRC Press, New York.

Feng, H., & Yang, W. (2011). Ultrasonic processing. In: Zhang, H.Q., Barbosa-Canovas, G.V., Balasubramaniom, V.M., Patrick-Dunne, C., Farkas, D.F., Yuan, J.T.C. (Eds.), Nonthermal Processing Technologies For Food. Blackwell Publishing, USA, pp. 135-155.

Feng, H., Yang, W., & Hielscher, T. (2008). Power ultrasound. *Food Science and Technology International*, 14, 433.

Fernández A., Noriega E., & Thompson A., (2013). Inactivation of *Salmonella enterica* serovar Typhimurium on fresh produce by cold atmospheric gas plasma technology. *Food Microbiology,* 33, 24-29.

Fernández, A., Shearer, N., Wilson, D. R., & Thompson, A. (2012). Effect of microbial loading on the efficiency of cold atmospheric gas plasma inactivation of *Salmonella enterica* serovar Typhimurium. *International Journal of Food Microbiology*, 152, 175-180.

Fonseca, J. M., & Rushing, J. W. (2006). Effect of ultraviolet-C light on quality and microbial population of fresh-cut watermelon. *Postharvest Biology and Technology*, 40, 256-261.

Fox, P. F., Guinee, T. P., Cogan, T. M., & McSweeney, P .L. H. (2000). Microbiology of Cheese Ripening. U knjizi Fun-damentales of Cheese Science, An Aspen Publication, Aspen Publishers, Inc. Gaithersburg, Maryland, 206-232.

Franciosi, E., Settanni, L., Cologna, N., Cavazza, A., & Poznanski, E. (2011). Microbial analysis of raw cow's milk used for cheese-making: influence of storage treatments on microbial composition and other technological traits. *World Journal of Microbiology and Biotechnology*, 27, 171-180.

Franz C. M. A. P., Specht I., Cho Gyu-Sung, Graef V., & Stahl M. R. (2009). UV-C-inactivation of microorganisms in naturally cloudy apple juice using novel inactivation equipment based on Dean vortex technology. *Food Control,* 20, 1103-1107.

Franzetti, L., & Scarpellini, M. (2007). Characterization of *Pseudomonas* spp. isolated from foods. *Annals of Microbiology*, 57, 39–47.

Fridman, A., & Kennedy, L. (2004). Plasma physics and engineering. New York: Taylor & Francis.

Gallego-Juárez, J.A., Riera, E., Blanco, S.F., Rodríguez-Corral, G., Acosta-Aparicio, V.M., & Blanco, A. (2007). Application of high-power ultrasound for dehydration of vegetables: processes and devices. *Drying Technolody*, 25, 1893-1901.

Gammariello, D., Conte, A., Buonocore, G. G., & Del Nobile, M. A. (2011). Bio-based nanocomposite coating to preserve quality of Fior di latte cheese. *Journal of Dairy Science*, 94, 5298-5304.

Gammariello, D., Conte, A., Di Giulio, S., Attanasio, M., & Del Nobile, M. A. (2009). Shelf-life of Stracciatella cheese under modified atmosphere packaging. *Journal of Dairy Science*, 92, 483-490.

Gammariello, D., Di Giulio, S., Conte, A., & Del Nobile, M. A. (2008). Effects of natural compounds on microbial safety and sensory quality of Fior di latte cheese, a typical Italian cheese. *Journal Dairy Science*, 91, 4138–4146.

Gera, N., & Doores, S. (2011). Kinetics and mechanisms of bacterial inactivation by ultrasound waves and sono-protective effect of milk components. *Journal of Food Science*, 76, M111-M119.

Gómez, P. L., Alzamora, S. M., Castro, M. A., & Salvatori, D. M. (2010). Effect of ultraviolet-C light dose on quality of cut-apple: Microorganism, color and compression behavior. *Journal of Food Engineering*, 98, 60-70.

Gómez-López, V. M., Devlieghere, F., Bonduelle, V., & Debevere, J. (2005a). Factors affecting the inactivation of microorganisms by intense light pulses. *Journal of Applied Microbiology*, 99, 460-470.

Gómez-López, V. M., Devlieghere, F., Bonduelle, V., & Debevere, J. (2005b). Intense light pulses decontamination of minimally processed vegetables and their shelf-life. *International Journal of Food Microbiology*, 103, 79-89.

Gómez-López, V. M., Ragaert, P., Debevere, J., & Devlieghere, F. (2007). Pulsed light for food decontamination: A review. *Trends in Food Science & Technology*, 18, 464–473.

Gómez-López, V. M., Koutchma, T. & Linden, K. (2012). Ultraviolet and pulsed light processing of fluid foods. In *Novel Thermal and Non-Thermal Technologies for Fluid Foods* (eds P.J. Cullen, B. Tiwari and V. Valdramidis). Academic Press, San Diego, CA, pp. 185–223.

Grecz, N., Rowley, D. B. & Matsuyama, A. (1983). The action of radiation on bacteria and viruses, in *Preservation of foods by Ionizing Radiation*, vol. 2. CRC Press, Boca, Raton, FI.

Green, A. K., Guzel-Seydim, Z. B., & Seydim, A. C. (2012). Chemical and physical properties of ozone. In Ozone in Food Processing ed. O'Donnel, C., Tiwary, B. K., Cullen, P. J. and Rice, R. G. pp. 26–28. UK: Blackwell Publishing Ltd.

Guana, W., Fanb, X., & Yana, R., (2012). Effects of UV-C treatment on inactivation of *Escherichia coli* O157:H7, microbial loads, and quality of button mushrooms. *Postharvest Biology and Technology,* 64, 119-125.

Guerrero-Beltrán, J. A., & Barbosa-Cánovas, G. V. (2004). Review: advantages and limitations on processing foods by UV light. *Food Science and Technology International,* 28, 137-148.

Guiseppi-Elie, A., Choi, S-H. & Geckeler, K.E. (2009). Ultrasonic processing of enzymes: Effect on enzymatic activity of glucose oxidase. *Journal of Molecular Catalysis*, 58, 118-123

Guneser, O., & Karagul Yuceer, Y. (2012). Effect of ultraviolet light on water- and fat-soluble vitamins in cow and goat milk. *Journal of Dairy Science.* 95, 6230–6241.

Gupta, S. (2011). Milk goes ultraviolet, *New Scientist*, 210, 19.

Hansen, D., & Larsen, B.S. (2007). Reduction of *Campylobacter* on chicken carcasses by SonoSteam® treatment. Proceeding of European Congress of Chemical Engineering (ECCE-6), Copenhagen, and 16–20 September 2007.

Haughton, P., Lyng, J., Morgan, D., Cronin, D., Noci, F., Fanning, S., & Whyte, P. (2012). An evaluation of the potential of high-intensity ultrasound for improving the microbial safety of poultry. *Food Bioprocess Technol*ogy, 5, 992-998.

Haughton, P. N., Lyng, J. G., Morgan, D. J., Cronin, D. A., Fanning, S., & Whyte, P. (2011). Efficacy of high-intensity pulsed light for the microbiological decontamination of chicken, associated packaging, and contact surface. *Foodborne Pathogens and Disease*, 8, 109-117.

Hillegas, S. L., & Demirci, A. (2003). Inactivation of *Clostridium sporogenes* in clover honey by pulsed UV-light treatment. *Agricultural Engineering International*, V. Manuscript FP 03 009.

Hiramoto, T. (1984). Method of sterilization. US Patent number 4464336.

Hoornstra, E., de Jong, G., & Notermans, S. (2002). Preservation of vegetables by light. In Conference frontiers in microbial fermentation and preservation (pp. 75–77). The Netherlands: Wageningen.

Huo, J. X., Bai, C. Y., Guo, L. H., & Zhao, Z. (2013). Effect of electron beam irradiation on the shelf-life of mozzarella cheese. *International Journal of Dairy Technology*, 66, 3.

Ignat, A., Manzocco, L., Maifreni, M., Bartolomeoli, I., & Nicoli, M. C. (2014). Surface decontamination of fresh-cut apple by pulsed light: effects on structure, colour and sensory properties. *Postharvest Biology and Technology*, 91, 122-127.

Incoronato, A. L., Conte, A., Buonocore, G. G., & Del Nobile, M. A. (2011). Agar hydrogel with silver nanoparticles to prolong the shelf life of Fior di Latte cheese. *Journal of Dairy Science*, 94, 1697-1704.

Innocente, N., Segat, A., Manzocco, L., Marino, M., Maifreni, M., Bortolomeoli, I., Ignat, A., & Nicoli, M. C. (2014). Effect of pulsed light on total microbial count and alkaline phosphatase activity of raw milk. *International Dairy Journal,* 39, 108-112.

International Consultative Group on Food Irradiation (ICGFI). Facts about food irradiation. (1999).

International Consultative Group on Food Irradiation. (1991). Facts about food irradiation. (set of 14 fact sheets covering all aspects of food irradiation issued as public information). ICGFI Fact Series 1- 14. IAEA, Vienna.

Isbary, G., Shimizu, T., Li, Y. F., Stolz, W., Thomas, H., & Morfill, G. (2013). Cold atmospheric plasma devices for medical issues. *Expert Rev Med Devices*, 10, 367-77.

Isohanni, P. M. I., & Lyhs, U. (2009). Use of ultraviolet irradiation to reduce *Campylobacter jejuni* on broiler meat. *Poultry Science*, 88, 661-668.

Izquier, A., & Gómez-López, V. M. (2011). Modeling the pulsed light inactivation of microorganisms naturally occurring on vegetable substrates. *Food Microbiology*, 28, 1170–1174.

James, C., James, S. J., Hannay, N., Purnell, G., Barbedo-Pinto, C., Yaman, H., Araujo, M., Gonzalez, M. L., Calvo, J., Howell, M., & Corry, J. E. L. (2007). Decontamination of poultry carcasses using steam or hot water in combination with rapid cooling, chilling or freezing of the carcass surface. *International Journal of Food Microbiology*, 114, 195-203.

Jay, J. M. (1995). Radiation preservation of foods and nature of microbial radiation resistance. In *Modern Food Microbiology* (ed. J.M. May). Springer New York, pp. 304–327.

Jiang, T., Jahangir, M. M., Jiang, Z., Lu, X., & Ying, T. (2010). Influence of UV-C treatment on antioxidant capacity, antioxidant enzyme activity and texture of postharvest shiitake (Lentinus edodes) mushrooms during storage. *Postharvest Biology and Technology*, 56, 209-215.

Jofré A., Aymerich T., & Garriga M. (2008). Assessment of the effectiveness of antimicrobial packaging combined with high pressure to control Salmonella sp. in cooked ham. *Food Control*, 19, 634-638.

Joint FAO/IAEA/WHO Study Group. Wholesomeness of irradiated food. WHO Technical Series 659. Geneva: WHO; 1981.

Juraga, E., Salamon, B. S. & Herceg, Z. (2011). Application of high intensity ultrasound treatment on *Enterobacteriaceaee* count in milk. *Mljekarstvo*, 61, 125-134.

Kampelmacher, E.H. (1984). Irradiation of food: a new technology for preserving and ensuring the hygiene of foods. *Fleisch-wirtschaft* 64, 322–327. In: Farkas J. (1998). *International Journal of Food Microbiology,* 44,189-204.

Kanatt S. R., Chander R., & Sharma A. (2005). Effect of radiation processing on the quality of chilled meat products. *Meat Science*, 69, 269-275.

Kentish, S., & Ashokkumar, M. (2011). The physical and chemical effects of ultrasound. In: Feng, H., Barbosa-Cánovas, G.V., Weiss, J. (Eds.), *Ultrasound Technologies for Food and Bioprocessing*. Springer, London, pp. 1–12.

Keyser, M., Müller, I. A., Cilliers, F. P., Nel, W., & Gouws, P. A. (2008). Ultraviolet radiation as a non-thermal treatment for the inactivation of microorganisms in fruit juice. *Innovative Food Science & Emerging Technologies*, 9, 348-354.

Kim, K., Kim, G., Hong, Y. C., & Yang, S. S. (2010). A cold micro plasma jet device suitable for bio-medical applications. *Microelectronic Engineering,* 87, 1177-1180.

Kinder, A. A. (1986). Potential application of ionizing radiation in postharvest handing of fresh fruits and vegetables. *Food Technology*, 40, 117-121.

Kindstedt, P., Carić, M., & Milanović, S. (2004). *Pasta-filata cheeses.* In P. Fox, P. L. H. McSweeney, T. M. Cogan, & T. P. Guinee (Eds.) (3rd ed.).Cheese: Chemistry, physics and microbiology: Vol. 2. Major cheese groups Elsevier Academic Press.

Klockow, P., & Keener, K. (2009). Safety and quality assessment of packaged spinach treated with a novel ozone-generation system. *LWT Food Science and Technology*, 42, 1047-53.

Klockow, P. A. & Keener, K. M. (2009). Safety and quality assessment of packaged spinach treated with a novel ozone-generation system. *LWT–Food Science and Technology*, 42, 1047-1053.

Knorr, D., Zenker, M., Heinz, V. & Lee, D. U. (2004). Applications and potential of ultrasonics in food process-ing. *Trends in Food Science and Technology*, 15, 261- 266.

Komolprasert, V., & Morehouse, K. (2004). Irradiation of Food and Packaging, pp. 6. Washington: American Chemical Society.

Koutchma, T. (2009). Advances in ultraviolet light technology for non-thermal processing of liquid foods, *Food and Bioprocess Technology*, 2, 138-155.

Koutchma, T., Parisi, B., & Patazca, E. (2007). Validation of UV coiled tube reactor for fresh juices. *Journal of Environmental Engineering and Science*, 6, 319-328.

Krishnamurthy, K., Demirci, A. & Irudayaraj, J. (2008). Inactivation of *Staphylococcus aureus* in milk and milk foam by pulsed UV-light treatment and surface response modeling. *Transactions of the ASABE*, 51, 2083-2090.

Krishnamurthy, K., Demirci, A., & Irudayaraj, J. (2004). Inactivation of *Staphylococcus aureus* by pulsed UV-light sterilization. *Journal of Food Protection*, 67, 1027-1030.

Krishnamurthy, K., Demirci, A., & Irudayaraj, J. M. (2007). Inactivation of *Staphylococcus aureus* in milk using flow-through pulsed UV light treatment system. *Journal of Food Science*, 72, M233-M239.

Lagunas-Solar, M. C., Piña, C., MacDonald, J. D., & Bolkan, L. (2006). Development of pulsed UV light processes for surface fungal disinfection of fresh fruits. *Journal of Food Protection*, 69, 376-384.

Langeveld, L. P. M., & Cuperus, F. (1976). The Relation Between Temperature and Growth Rate in Pasteurized Milk of Difference Types of Bacteria Which are Important to the Deteriation of that Milk. *Nethelands Milk Dairy Journal,* 34, 106-125.

Laroussi, M., & Leipold, F. (2004). Evaluation of the roles of reactive species, heat, and UV radiation in the inactivation of bacterial cells by air plasmas at atmospheric pressure. *International Journal of Mass Spectrometry*, 233, 1-3 81-86.

Laroussi, M., Kong, M. G., Morfill, G., & Stolz, W., (2012) editors. Plasma medicine. Cambridge: Cambridge University Press.

Larsen, H.D., & Jørgensen, K. (1997). The occurrence of *Bacillus cereus* in Danish pasteurized milk. *International Journal of Food Microbiology 34*, 179-186.

Lee, H., & Feng, H., (2011). Effect of power ultrasound on food quality. In: Feng, H., Barbosa- Cánovas, G.V., Weiss, J. (Eds.), *Ultrasound Technologies for Food and Bioprocessing*. Springer, London, pp. 559–582.

Leighton, T. (1994). The acoustic bubble. Academic Press, London.

Leighton, T. (2007). What is ultrasound? *Progress in Biophysics and Molecular Biology*, 93, 3–83.

Liberty, J. T., Dickson, D. I., Achebe, A. E., & Salihu, M. B. (2013). An Overview of the Principles and Effects of Irradiation on Food Processing & Preservation. *International Journal of Multidisciplinary and Current Research*, ISSN: 2321-3124.

Lillard, H.S., (1994). Decontamination of poultry skin by sonication. *Food Technol*ogy, 48, 72-73.

López-Malo, A., & Palou, E. (2005). Ultraviolet light and food preservation. In: Barbosa-Cánovas, G.; Tapia, M. S.; Cano, P. Novel food processing technologies. New York: CRC. Chap. 18.

Loretz, M., Stephan, R., & Zweifel, C. (2010). Antimicrobial activity of decontamination treatments for poultry carcasses. A literature survey. *Food Control,* 21, 791-804.

Lu, X. P., Ye, T., Cao, Y. G., Sun, Z. Y., Xiong, Q., Tang, Z. Y., Xiong, Z. L., Hu, J., Jiang, Z. H., & Pan, Y. (2009). The roles of the various plasma agents in the inactivation of bacteria. *Journal of Applied Physics*, 104, 053309

Mahmoud, B. S. M. (2009a). Effect of X-ray treatments on inoculated *Escherichia coli* O157:H7, *Salmonella enterica, Shigella flexneri and Vibrio parahaemolyticus* in ready-to-eat shrimp. *Food Microbiology*, 26, 860-864.

Mahmoud, B. S. M. (2009b). Inactivation effect of X-ray treatments on *Cronobacter* species (*Enterobacter sakazakii*) in tryptic soy broth, skim milk, low-fat milk and whole-fat milk. *Letters in Applied Microbiology*, 49, 562-567.

Mahmoud, B. S. M. (2010). Inactivation of *Escherichia coli* O157:H7*, Listeria monocytogenes, Salmonella enterica* and *Shigella flexneri* on whole Roma tomatoes by X-ray. *Food Microbiology*, 27, 1057-1063.

Mahmoud, B. S. M., Bachman, G., & Linton, R. H. (2010). Inactivation of *Escherichia coli* O157:H7, *Listeria monocytogenes, Salmonella enterica* and *Shigella flexneri* on spinach leale by X-ray. *Food Microbiology*, 27, 24–28.

Mahmoud, B. S. M., Coker, R., & Su, Y. C. (2012). Reduction in *Listeria monocytogenes* and spoilage bacteria on smoked catfish using X-ray treatments. *Letters in Applied Microbiology*, 54, 524-529.

Mahmoud, B. S. M., Nannapaneni, R., Chang, S., Wu, Y., & Coker, R., (2016). Improving the safety and quality of raw tuna fillets by X-ray irradiation. *Food Control*, 60, 569-574.

Manas, P., Pagan, R., Raso, J., Sala, F. J., & Condon, S., (2000a). Inactivation of *Salmonella Typhimurium,* and *Salmonella Senftenberg* by ultrasonic waves under pressure. *Journal of Food Protection* 63, 451-456.

Manzocco L., Da Pieve S., Bertolini A., Bartolomeoli I., Maifreni M., Vianello A., & Nicoli M. C. (2011). Surface decontamination of fresh-cut apple by UV-C light exposure: Effects on structure, colour and sensory properties. *Postharvest Biology and Technology,* 61, 165-171.

Manzocco L., Panozzo A., & Nicoli M. C. (2013). Effect of pulsed light on selected properties of egg white. *Innovative Food Science and Emerging Technologies,* 18, 183-189.

Manzocco, L., Panozzo, A., & Nicoli, M. C. (2012). Effect of ultraviolet processing on selected properties of egg white. *Food Chemistry* 135(2), 522-527.

Manzocco, L., Plazzotta, S., Maifreni, M., Calligaris, S., Anese, M., & Nicoli, M. C. (2015). Impact of UV-C light on storage quality of fresh-cut pineapple in two different packages. *LWT -Food Science and Technology,* doi: 10.1016/j.lwt.2015.10.007.

Marquenie, D., Geeraerd, A. H., Lammertyn, J., Soontjens, C., Van Impe, J. F., Michiels, C. W., & Nicolaï, B. M. (2003). Combinations of pulsed white light and UV-C or mild heat treatment to inactivate conidia of *Botrytis cinerea* and *Monilia fructigena*. *International Journal of Food Microbiology*, 85, 185-196.

Martin, N. H., Murphy, S. C., Ralyea, R. D., Wiedmann, M., & Boor, K.J. (2011). When cheese gets the blues: *Pseudomonas fluorescens* as the causative agent of cheese spoilage. *Journal of Dairy Science*, 94, 3176-3183.

Mason, T. J. (1998). Power ultrasound in food processing-The way. *Ultrasound in food processing*, 105.

Mason, T. J., Paniwnyk, L. & Lorimer, J. P. (1996) The uses of ultrasound in food technology. *Ultrasonics Sono-chemistry*, 3, 253-260.

Mastromatteo, M., Conte, A., Lucera, A., Saccotelli, M. A., Buonocore, G. G., Zambrini, A. V., & Del Nobile, M.A. (2015 a). Packaging solutions to prolong the shelf life of Fiordilatte cheese: Bio-based nanocomposite coating and modified atmosphere packaging. *LWT-Food Science and Technology*, 60, 230-237.

Mastromatteo, M., Lucera, A., Esposto, D. Conte, A., Faccia, M., Zambrini, A. V., Del Nobile, M. A., (2015 b). Packaging optimization to prolong the shelf life of fiordilatte cheese . *Journal of Dairy Research*, 82, 143-151.

Matak, K. E., Churey, J. J., Worobo, R. W., Summer, S. S., Hovingh, E., Hackney, C. R., & Pierson, M. D. (2005). Efficacy of UV light for the reduction of *Listeria monocytogenes* in goat's milk. *Journal of Food Protection*, 68, 2212-2216.

McClements, D. J. (1995). Advances in the application of ultrasound in food analysis and processing. *Trends in Food Science and Technology*, 6, 293-299.

McDonald, K. F., Curry, R. D., Clevenger, T. E. K. U., Eisenstark, Abraham, Golden, Jeffrey, Morgan, Robert D., (2002). A comparison of pulsed and continuous ultraviolet light sources for the decontamination of surfaces. *IEEE Transactions on Plasma Science,* 28, 1581-1587.

McDonnell, C. K., Allen, P., Chardonnereau, F. S., Arimi, J. M., & Lyng, J. G. (2014). The use of pulsed electric fields for accelerating the salting of pork. *LWT - Food Science and Technology,* 59, 1054-1060.

Miller, B. M., Sauer, A. & Moraru, C. I. (2012). Inactivation of *Escherichia coli* in milk and concentrated milk using pulsed-light treatment. *Journal of Dairy Science*, 95, 5597-5603.

Misra, N. N., Tiwari, B. K., Raghavarao, K. S. M. S. & Cullen, P. J. (2011). Non-thermal plasma inactivation of food-borne pathogens. *Food Engineering reviews*, 3, 159-170.

Moisan, M., Barbeau, J., Crevier, M. C., Pelletier, J., Philip, N., & Saoudi, B. (2002). Plasma sterilization. Methods and mechanisms. *Pure and applied chemistry*, 74, 3, 349-358.

Molins R. (2001). Food irradiation: principles and applications. Books. Google.com

Montgomery, J. L., Parrish, Jr. F. C., Olsonb, D. G., Dicksonb, J. S., & Niebuhrc, S. (2003). Storage and packaging effects on sensory and color characteristics of ground beef. *Meat Science*, 64, 357-363.

Morales, P., Garcia, E. F., & Nunez, M. (2005). Volatile compounds produced in cheese by *Pseudomonas* strains of dairy origin belonging to six different species. *Journal of Agriculture and Food Chemistry*, 53, 6835-6843.

Moraru, C. I. (2011). High-intensity pulsed light food processing. In *Alternatives to Conventional Food Processing* (ed. A. Procter). The Royal Society of Chemistry, Cambridge, UK, pp. 367–386.

Morild, R. K., Christiansen, P., Sørensen, A. H., Nonboe, U., & Aabo, S. (2011). Inactivation of pathogens on pork by steam-ultrasound treatment. *Journal of Food Protection*, 5, 769-775.

Musavian, H. S, Butt, T. M., Larsen, A. B., & Krebs, N. (2015). Combined Steam-Ultrasound Treatment of 2 Seconds Achieves Significant High Aerobic Count and *Enterobacteriaceaeceae* Reduction on Naturally Contaminated Food Boxes, Crates, Conveyor Belts, and Meat Knives. *Journal of Food Protection*, 78, 430-435.

Musavian, H. S., Krebs, N. H., Nonboe, U., Corry, J. E. L., & Purnell, G. (2014). Combined steam and ultrasound treatment of broilers at slaughter: A promising intervention to significantly reduce numbers of naturally occurring *Campylobacters* on carcasses. *International Journal of Food Microbiology,* 176, 23-28.

Muthukumaran, S., Kentish, S. E., Stevens, G. W., & Ashokkumar, M. (2006). Application of ultrasound in membrane separation processes: A review. *Reviews in chemical engineering*, 22(3), 155–194.

Nardiello, D., Conte, A., Natale, A., Lucera, A., Palermo, C., Centonze, D., & Del Nobile, M. A. (2014). Effects of different packaging systems on microbiological, sensory and peptide profile in fiordilatte cheese. *Food Research International*, 62, 628-636.

Niemira, B. A. (2012). Cold plasma decontamination of foods. In M. P. Doyle, & T. R. Klaenhammer (Eds.), *Annual review of food science and technology*, Vol. 3. (pp. 125–142) Palo Alto, USA Annual Reviews.

Oehmigen, K., Hahnel, M., Brandenburg, R.,Wilke, Ch., Weltmann, K. D. & Woedtke, T. (2010). The role of acidification for antimicrobial activity of atmospheric pressure plasma in liquids. *Plasma Processes Polymers*, 7, 250-257.

Oms-Oliu, G., Aguiló-Aguayo, I., Martín-Belloso, O., & Soliva-Fortuny, R. (2010 a). Effects of pulsed light treatments on quality and antioxidant properties of fresh-cut mushrooms (Agaricus bisporus). *Postharvest Biology and Technology*, 56, 216-222.

Oms-Oliu, G., Martín-Belloso, O. & Soliva-Fortuny, R. (2010 b) Pulsed light treatments for food preservation. A review, *Food and Bioprocess Technology*, 3, 13-23.

Oner, M. E., & Wall, M. M. (2013). Quality of fresh-cut purple-fleshed sweet potatoes after X-ray irradiation treatment and refrigerated storage. *International Journal of Food Science and Technology*, 48, 2064-2070.

Oommen, B. S., McMahon, D. J., Oberg, C. J., Broadbent, J. R., & Strickland, M. (2002). Proteolytic specificity of *Lactobacillus delbrueckii* subsp. *bulgaricus* influences functional properties of Mozzarella cheese. *Journal of Dairy Science*, 85, 2750–2758.

Orlowska, M., Koutchma, T., Grapperhaus, M., Gallagher J., Schaefer R., & Defelice, C. (2013). Continuous and pulsed ultraviolet light for non-thermal treatment of liquid foods. Part 1: effects on quality of fructose solution, apple juice, and milk. *Food and Bioprocess Technology*, 6, 1580-1592.

Ozer, N. P., & Demirci, A. (2006). Inactivation of *Escherichia coli* O157:H7 and *Listeria monocytogenes* inoculated on raw salmon fillets by pulsed UV-light treatment. *International Journal of Food Science & Technology,* 41, 354–360.

Pagan, R., Manas, P., Alvarez, I., & Condon, S. (1999). Resistance of *Listeria monocytogenes* to ultrasonic waves under pressure at sub-lethal (manosonication) and lethal (manothermosonication) temperatures. *Food Microbiology*, 16, 139-148.

Palgan, I., Caminiti, I. M., Muñoz, A., Noci, F., Whyte, P., Morgan, D. J., Cronin, D. A., & Lyng, J. G. (2011). Effectiveness of High Intensity Light Pulses (HILP) treatments for the control of *Escherichia coli* and *Listeria innocua* in apple juice, orange juice and milk. *Food Microbiology*, 28, 14-20.

Panozzo, A., Manzocco, L., Lippe, G., & Nicoli, M. C. (2016). Effect of pulsed light on structure and immunoreactivity of gluten. *Food Chemistry* 194, 366-372.

Patist, A., & Bates, D. (2008). Ultrasonic innovations in the food industry: From the laboratory to commercial production. *Innovative Food Science & Emerging Technologies*, 9, 147-154.

Pelletier, J. (1992). Sterilisation by plasma processing. *Agressologie,* 33, 105-110.

Philips, L. (2006). Ultraviolet purification and application information (Lighting Brochure). Philips Lighting, The Netherlands, p. 18.

Piyasena, P., Mohareb, E. & Mckellar, R. C. (2003) Inactivation of microbes using ultrasound: A review. *International Journal of Food Microbiology*, 87, 207-216.

Polydera, A. C., Stoforos, N. G., & Taoukis, P. S. (2005). Quality degradation kinetics of pasteurised and high pressure processed fresh Navel orange juice: Nutritional parameters and shelf life. *Innovative Food Science and Emerging Technologies,* 6, 1-9.

Ramos-Villarroel, A., Martín-Belloso, O., & Soliva-Fortuny, R. (2011). Bacterial inactivation and quality changes in fresh-cut avocado treated with intense light pulses. *European Food Research and Technology*, 233, 395-402.

Ramos-Villarroel, A. Y., Aron-Maftei, N., Martín-Belloso, O., & Soliva-Fortuny, R., (2012a). Influence of spectral distribution on bacterial inactivation and quality changes of fresh-cut watermelon treated with intense light pulses. *Postharvest Biology and Technology*, 69, 32-39.

Ramos-Villarroel, A. Y., Aron-Maftei, N., Martín-Belloso, O., & Soliva-Fortuny, R., (2012b). The role of pulsed light spectral distribution in the inactivation of Escherichia coli and Listeria innocua on fresh-cut mushrooms. *Food Control*, 24, 206-213.

Raso, J., & Barbosa-Canovas, G. V. (2003). Nonthermal preservation of foods using combined processing tech- niques. *Critical Reviews in Food Science and Nutrition*, 43, 265-285.

Raso, J., Palo, A., Pagan, R., & Condon, S., (1998a). In activation of *Bacillus subtilis* spores by combining ultrasonic waves under pressure and mild heat treatment. *Journal of Applied Microbiology,* 85, 849-854.

Rastogi, N. K. (2010). Opportunities and challenges in nonthermal processing of foods. In: Passos, M.L., Ribeiro, C.P. (Eds.), *Innovation in Food Engineering: New Techniques and Products*. CRC Press, USA, pp. 4–41.

Raviyan, P., Zhang, Z. & Feng, H. (2005). Ultrasonication for tomato pectinmethylesterase inactivation: Effect of cavitation intensity and temperature on inactivation. *Journal of Food Engineering*, 70, 189-196.

Reinemann, D. J, Gouws, P., Chillier, T., Houck, K., & Bishop, J. R. (2006). New methods of UV treatment of milk for improved food safety and product quality. Annual International Meeting, American Society of Agricultural and Biological Engineers (ASABE), Paper Number: 066088, 1-9.

Rico, D., Martin-Diana, A. B., Barat, J. M., & Barry-Ryan, C. (2007). *Extending and measuring the quality of fresh-cut fruit and vegetables*: A review. *Trends in Food Science & Technology*, 18, 373–386.

Roberts, T., & Weese, J. (1995). *Food Irradiation.* ACES Publications

Roberts, P., & Hope, A. (2003). Virus inactivation by high intensity broad spectrum pulsed light. *Journal of Virological Methods*, 110, 61-65.

Ross, A. I. V., Griffiths, M. W., Mittal, G. S., & Deeth, H. C. (2003). Combining non-thermal technologies to control foodborne microorganisms. *International Journal of Food Microbiology*, 89 125-138.

Rossito, P. V., Cullor, J. S., Crook, J., Parko, J., Sechi, P. & Cenci-Goga, B. T. (2012). Effects of UV Irradiation in a Continuous Turbulent Flow UV Reactor on Microbiological and Sensory Characteristics of Cow's Milk. *Journal of Food Protection*, 75, 2197-2207.

Roth, S., Feichtinger, J., & Hertel, C. (2010). Characterization of *Bacillus subtilis* spore inactivation in low pressure, low temperature gas plasma sterilization processes. *Journal of applied microbiology,* 108, 521-531.

Rowan, N. J., MacGregor, S. J., Anderson, J. G., Fouracre, R. A., McIlvaney, L., & Farish, O. (1999). Pulsed-light inactivation of food-related microorganisms. *Applied and Environmental Microbiology*, 65, 1312-1315.

Şahin Ercan, S. & Soysal, Ç. (2011). Effect of ultrasound and temperature on tomato peroxidase. *Ultrasonics Sonochemistry*, 18, 689-695.

Sala, F.J., Burgos, J., Condon, S., Lopez, P. & Raso, J. (1995) Effect of heat and ultrasound on microorganisms and enzymes. In: Gould, G.W., Ed., *New Methods of Food Preservation*, Blackie Academic and Professional, Lon- don, 176-204.

Sale, A. J. H., Gould, G. W., & Hamilton, W. A. (1970). Inactivation of bacterial spores by hydrostatic pressure. *Journal of General Microbiology*, 60, 323-334.

Sale, A.J.H., & Hamilton, W. A. (1968). Effects of high electric fields on micro-organisms: III. Lysis of erythrocytes and protoplasts. *Biochemica et Biophysica Acta*, 63, 37-43.

Samaržija, D., Zamberlin, Š., & Pogačić, T. (2012). Psychrotrophic bacteria and milk and dairy products quality. Review. *Mljekarstvo* 62 (2), 77-95.

Santana, E. H. W., de. Beloti, V., Müller, E. E., Ferreira, M. A. de., Morales, L.B.de., Pereira, M. S., & Gusmão, V. V. (2004). Milk contamination in different points of

dairy process. ii) mesophilic, psychrotrophic and proteolytic microorganisms. *Semina: Ciências Agrárias; Londrina 25*, 349-358.

Sastry, S.K.,Datta, A.K. & Worobo, R. W. (2000). Ultraviolet light, *Journal of Food Safety*, 65, 90–92.

Schwabedissen, A., Lacinski, P., Chen, X. & Engemann, J. (2007). PlasmaLabel–a new method to disinfect goods inside a closed package using dielectric barrier discharges. *Contribution Plasma Physics*, 47, 551-558.

Serrano, L. E., Murano, E. A., Shenoy, K., & Olson, D. G. (1997). *D*-values of *Salmonella enteritidis* isolates and quality attributes of shell eggs and liquid whole eggs treated with irradiation. *Poultry Science*, 76, 202-206.

Shama, G. (1999). Ultraviolet light. In R. K. Robinson, C. Batt, & P. Patel (Eds.), Encyclopedia of food microbiology. (pp. 2208– 2214). London: Academic.

Shama, G. (2007). Process challenges in applying low doses of ultraviolet light to fresh produce for eliciting beneficial hermetic responses. *Postharvest Biology and Technology*, 44, 1–8.

Sharma, R. R., & Demirci, A. (2003). Inactivation of *Escherichia coli* O157:H7 on inoculated alfalfa seeds with pulsed ultraviolet light and response surface modeling. *Journal of Food Science*, 68, 1448-1453.

Sheldon, S. A., Jones, A. D., & Shibamoto, T. (1988). Photochemical products of a cysteine/D-glucose browning model system. Journal of Agriculture and Food Chemistry, 36, 604-606.

Siddiqui, M. W., Chakraborty, I., Ayala-Zavala, J. F., & Dhua, R. S. (2011). Advances in minimal processing of fruits and vegetables: a review. *Journal of Scientific and Industrial Research*, 70, 823-834.

Singh, S. P., & Pal, R. K. (2009). Ionizing radiation treatment to improve postharvest life and maintain quality of fresh guava fruit. *Radiation Physics and Chemistry*, 78, 135-140.

Sinigaglia, M., Bevilacqua, A., Corbo, M. R., Pati, S., & Del Nobile, M. A. (2008). Use of active compounds for prolonging the shelf life of Mozzarella cheese. *International Dairy Journal*, 18, 624–630.

Smith, D. P. (2011). Effect of ultrasonic marination on broiler breast meat quality and Salmonella contamination. *International Journal of Poultry Science*, 10, 757-759.

Smith, W. L., Lagunas-Solar, M. C., & Cullor, J. S. (2002). Use of pulsed ultraviolet laser light for the cold pasteurization of bovine milk. *Journal of Food Protection*, 65, 1480-1482.

Soliva-Fortuny, R. C., & Martín-Belloso, O. (2003). New advances in extending the shelf-life of fresh-cut fruits: a review. *Trends in Food Science & Technology*, 14, 341-353.

Soncini, G., Marchisio, E., & Cantoni, C. (1998). Causes of chromatic alterations in Mozzarella cheese. *Industrie Alimentari,* 37, 850-855.

Sonenshein, A. L. (2003). Killing of *Bacillus* spores by high-intensity ultraviolet light. In sterilization and decontamination using high-energy light. Xenon Corporation, Woburn, Mass. Pp. 15-19.

Song, H. P., Kim, B., Choe, J. H., Jung, S., Moon, S. Y., Choe, W. & Jo, C. (2009). Evaluation of atmospheric pressure plasma to improve the safety of sliced cheese and ham inoculated by 3-strain cocktail *Listeria monocytogenes*. *Food Microbiology*, 26, 4, 432-436.

Song, H. P., Kim, B., Choe, J. H., Jung, S., Moon, S. S., Choe, W. & Jo, C. (2009). Evaluation of atmospheric pressure plasma to improve the safety of sliced cheese and ham inoculated by 3-strain cocktail *Listeria monocytogenes*. *Food Microbiology,* 26, 432-436.

Sosnin, E. A., Oppenländer, T., & Tarasenko, V. F. (2006). Applications of Capacitive and Barrier Discharge Excilamps in Photoscience. *Journal of Photochemistry and Photobiology C: Photochemistry Reviews*, 7, 145-163.

Spano, G. E., Goffredo, L., Beneduce, D., & Tarantino, A. (2003). Fate of Escherichia coli O157:H7 during the manufacture of Mozzarella cheese. *Letters in Applied Microbiology*, 36, 73-76.

Stewart, Eileen M. (2004). Food Irradiation: more pros and cons? *Biologist*, 51, 91-94.

Tahiri, I., Makhlouf, J., Paquin, P., & Fliss, I. (2006). Inactivation of food spoilage bacteria and *Escherichia coli* O157:H7 in phosphate buffer and orange juice using dynamic high pressure. *Food Research International*, 39, 98-105.

Takeshita, K., Shibato, J., Sameshima, T., Fukunaga, S., Isobe, S., Arihara, K., & Itoh, M. (2003). Damage of yeast cells induced by pulsed light irradiation. *International Journal of Food Microbiology*, 85, 151-158.

Tappi, S., Berardinelli, A., Ragni, L., Dalla Rosa, M., Guarnieri ,A., & Rocculi, P. (2014). Atmospheric gas plasma treatment of fresh-cut apples. *Innovative Food Science and Emerging Technologies*, 21, 114-122.

Tendero, C., Tixier, C., Tristant, P., Desmaison, J., & Leprince, P. (2006). Atmospheric pressure plasmas: a review. *Spectrochimica Acta B Atomic Spectroscopy*, 61, 2-30.

Tornaletti, S. (2005). Transcription Arrest at DNA Damage Sites. *Mutation Research*, 577, 131-145.

Tripathi, J., Chatterjee, S., Vaishnav, J., Variyar, P. S. & Sharma, A. (2013). Gamma irradiation increases storability and shelf life of minimally processed ready-to-cook (RTC) ash gourd (Benincasa hispida) cubes. *Postharvest Biology and Technoogy*, 76, 17-25.

Trojan, UV. (2012). UV Disinfection and Oxidation Treatment Systems–Trojan UV. Available on: http://www.trojanuv.com.

Trujillo, A. J., Capellas, M., Saldo, J., Gervilla, R., & Guamis, B. (2002). Applications of high-hydrostatic pressure on milk and dairy products: a review. *Innovative Food Science and Emerging Technologies*, 3, 295-307.

USDA, (2000). U.S. Food and Drug Administration Report. Kinetics of Microbial Inactivation for Alternative Food Processing Technologies: Ultrasound. Published June 2, 2000.

Valero, M., Recrosio, N., Saura, D., Muñoz, N., Marti, N., & Lizama, V. (2007). Effect of ultrasonic treatments in orange juice processing. *Journal of Food Engineering*, 80, 509-516.

Van Calenberg, S., Van Cleemput, O., Mondelaers, W., & Huyghebaert, A. (1999). Comparison of the effect of X-ray and electron beam irradiation on the microbiological quality of foodstuffs. *Lebensmittel-Wissenschaft & Technology*, 32, 372-376.

Van Calenberg, S., Vanhaelewyn, G., Van Cleemput, O., Callens, F., Mondelaers, W., & Huyghebaert, A. (1998). Comparison of the effect of X-ray and electron beam irradiation on some selected spices. *Lebensmittel-Wissenschaft & Technology*, 31, 252-258.

Vercet, A., Burgos, J., Crelier, S. & Lopez-Buesa, P. (2001). Inactivation of protease and lipase by ultrasound. *Innovation Food Science and Technologies*, 2, 139-150.

Villamiel, M., & de Jong, P. (2000). Inactivation of *Pseudomonas fluorescens* and *Streptococcus thermophilus* in trypticase soy broth and total bacteria in milk by continuous-flow ultrasonic treatment and conventional heating. *Journal of Food Engineering,* 45, 171-179.

Villamiel, M., Hamersveld, V. & De Jong, P. (1999). Review: Effect of ultrasound processing on the quality of dairy products. *Milchwissenschaft*, 54, 69-73.

Wang, R. X., Nian, W. F., Wu, H. Y., Feng, H. Q., Zhang, K., Zhang, J., Zhu, W. D., Becker, K. H., & Fang, J. (2012). Atmospheric-pressure cold plasma treatment of

contaminated fresh fruit and vegetable slices: inactivation and physiochemical properties evaluation. *The European Physical Journal D*, 66, 276.

Wang, T., MacGregor, S. J., Anderson, J. G., & Woolsey, G. A. (2005). Pulsed ultra-violet inactivation spectrum of *Escherichia coli*. *Water Research*, 39, 2921-2925.

Wekhof, A. (2000). Disinfection with flash lamps. *PDA Journal of Pharmaceutical Science and Technology*, 54, 264-276.

Wekhof, A., Trompeter, F. J., & Franken, O. (2001). Pulse UV disintegration (PUVD): a new sterilization mechanism for packaging and broad medical hospital applications. The first international conference on ultraviolet technologies. Washington D.C., USA.

Wilkinson, V. M., & Gould, G. W. (1996). Food irradiation. A reference guide (177).

Wordon, B. A., Mortimer, B. & McMast, L. D. (2011). Comparative real-time analysis of *Saccharomyces cerevisiae* cell viability, injury and death induced by ultrasound (20 kHz) and heat for the application of hurdle technology. *Food Research International*, 47, 134-139.

Wright, J. R., Sumner, S.S., Hackney, C. R., Pierson, M. D., & Zoecklein, B. W. (2000). Efficacy of Ultraviolet Light for reducing *Escherichia coli* O157:H7 in Unpasteurized Apple Cider. *Journal of Food Protection,* 63, 563-567.

Zenker, M., Heinz, V. & Knorr, D. (2003). Application of ultrasound-assisted thermal processing for preservation and quality retention of liquid foods. *Journal of Food Protection*, 66, 1642-1649.

Zheng, L. & Sun, D. W. (2006) Innovative applications of power ultrasound during food freezing processes-A review. *Trends in Food Science and Technology*, 17, 16-23.

Publications

Lacivita Valentina, Conte A., Manzocco L., Plazzotta S., Zambrini A. V., Del Nobile M. A., Nicoli M. C. (2016). Surface UV-C light treatments to prolong the shelf-life of Fiordilatte cheese. *Innovative Food Science & Emerging Technology,* 36, 150-155.